University of Alberta

Plasticity in the human adult nervous system:
Modulation and modification of spinal reflexes and corticospinal connection

by

Aiko Kido Thompson

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ABSTRACT

Neural plasticity is the ability of the nervous system to modulate or modify the connectivity between neurons in order to adapt to new conditions or environmental demands. Plasticity exists not only during the neural developmental period but throughout adult life. This thesis studied plasticity in the human adult central nervous system, in terms of modulation and modification of spinal reflexes and corticospinal connections.

In chapter 2, we found that spinal reciprocal inhibition is modulated from standing, to walking, to running, with a strong speed-dependence. Our present and other previous findings indicate that different spinal reflexes are differently modulated during different movements. In chapter 3, spinal reflexes were also examined on a much longer time frame. We showed for the first time that spinal reciprocal inhibition gradually decreases with age. Since excitatory reflexes also decrease with age, in healthy adult individuals a generally decreased excitability of spinal reflex pathways was suggested. Chronic changes in reciprocal inhibition after central nervous system (CNS) lesions were also studied in chapter 7.

Chapters 4, 5, and 6 studied neural plasticity induced by peripheral nerve stimulation in healthy subjects. Thirty minutes of common peroneal (CP) nerve stimulation at rest increased corticospinal excitability for an ankle flexor muscle (chapter 4). A similar stimulus-induced facilitatory effect could be obtained with short-term use of functional electrical stimulation (FES), in which muscle nerve stimulation is given with functionally appropriate timings during locomotion (chapter 5). Short-term effects of FES-assisted walking were mostly found as dramatic changes in corticospinal

excitability with minor changes in spinal reflexes (chapter 6). The series of studies indicated that corticospinal pathways are sensitive to changes in afferent and efferent inputs.

Chapter 8 reports three different cases of short- or long-term application of FES or FES-like stimulation. Three patients participated in our ongoing FES project for improving hemiplegic gait, and had foot drop that was treated by FES of the CP nerve. Present observations suggest that FES increases corticospinal excitability and/or induces cortical reorganization in patients with CNS lesions.

This thesis presents several pieces of evidence for plasticity in the human adult nervous system in health and disease.

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presented in B, D, F, and H. A and B: Slow walking (at 3 km/h), C and D: fast walking (at 6 km/h), E and F: slow running (i.e., jogging at 6 km/h), and G and H: running (at 9 km/h).

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MEP_{max} did not increase significantly at any time. Normalized soleus MEP_{max} (shown here) decreased following CP stimulation as did soleus MEP_h. D: the H-reflex amplitude did not increase with stimulation time. Data are means ± SE. Parabolas were computed by the least mean squares method to show the trends in A, B, and C that were significant.

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Fig. 6-1 Inhibition of the ongoing EMG activity by the antagonist muscle nerve stimulation. Filled circles show group means of normalized soleus inhibition (n=13) before, between, and after periods of walking with FES. Crosses represent group means of normalized TA inhibition (n=10). Error bars indicate ± SE. Thick horizontal bars indicate periods of walking.

Fig. 6-2 The soleus H_{max} decreased slightly but significantly after walking with FES. The change in the TA H_{max} was small and did not show any trends. A and B: the soleus H_{max} before (A) and after (B) walking with FES for 30 min in subject E.D. C: the group means ± SE of normalized soleus H_{max} (n=14) before, between, and after periods of walking with FES. D: the group means ± SE of the soleus background EMG during the H_{max} measurements. E: the group means ± SE of normalized TA H_{max} (n=11). Significant differences from the baseline tested by Student's t test are indicated by * ($P<0.05$) and ** ($P<0.01$).

Fig. 6-3 The short-latency reciprocal inhibition of the soleus H-reflex slightly changed after walking with FES. A: the group means ± SE of soleus H-reflexes conditioned by the CP nerve stimulation with short delays (n=8) before, between, and after periods of walking with FES. Note that a decrease of conditioned H-reflex implies an increase in inhibition. B: the group means ± SE of conditioned H-reflexes with long stimulus delays to test presynaptic inhibition (n=10). Thick horizontal bars indicate periods of walking. For a symbol, see Fig. 6-2.

Fig. 7-1 Reciprocal inhibition of the ongoing EMG activity following the antagonist muscle nerve stimulation (1.5 × MT) in a subject with MS (K.R.). A and C: the TA EMG and the rectified soleus EMG recorded in response to the CP nerve stimulation. The mean level of inhibition (mV) was measured for a 7-10 ms window (see short bar) including the peak depression in the rectified EMG. (The amount of inhibition is indicated as an arrow.) The stimulus artifact and contamination from the M-waves of nearby muscles have been removed for clarity. B and D: the soleus EMG and the rectified TA EMG activity after the tibial nerve stimulation. Note that there is little or no inhibition at the expected time (see horizontal bar). A longer latency excitation was sometimes seen, but has not been analyzed here. The dotted horizontal line indicates the background EMG level. Twenty sweeps were averaged for each part of the figure.

Fig. 7-2 Rectified EMG amplitudes for the soleus (A) and TA (B) MVC measured during standing are presented in relation to the time after onset of condition. Thick horizontal lines indicate the mean MVCs for healthy subjects (age 40-70), and dotted lines indicate lowest values in the control group, which were found in the oldest subject (age 70). No significant trends were found between MVC amplitudes and years after onset.

Fig. 7-3 Reciprocal inhibition of the ongoing EMG activity by 1.5 × MT of the antagonist muscle nerve stimulation in all subjects is shown as a function of the time after onset of condition. Thick horizontal straight lines indicate the mean values of normalized inhibition for healthy subjects (age 40-70). Filled symbols are for individuals who had stroke, and open symbols for individuals with other pathologies, such as SCI. A logarithmic curve was fitted to the group data and the Pearson's r value is presented for each condition. A: Inhibition of the soleus EMG activity by the CP nerve stimulation. The amount of inhibition, calculated as the difference between the background (BG) EMG and the mean rectified EMG of a selected 7-10 ms window, is normalized by the BG and averaged across different BG levels for each subject. B: Inhibition of the TA EMG activity by the tibial nerve stimulation. Negative values indicate excitation, instead of inhibition.

Fig. 7-4 Reciprocal inhibition of the ongoing EMG during 15% MVC of voluntary activation by the antagonist muscle nerve stimulation is presented in single subjects. A and B: The effect of weak CP nerve stimulation on the soleus EMG. Four sweeps were averaged. Stimulus intensities were 0.95 × MT for A, and 1.07 × MT for B. A period for inhibition is indicated by a thick horizontal grey bar. C: The effect of weak tibial nerve stimulation on the TA EMG. Stimulus intensities were 0.97 × MT for C, and 0.67 × MT

for D. A and C are from subject L.M. and B and D are from subject R.N.. E: Inhibition of the soleus EMG activity by the CP nerve stimulation is shown as a function stimulus intensity in subject R.N.. Note that the relative amount of inhibition (i.e., normalized by the background EMG) is used for the ordinate. Each point represents the mean of 4 responses. A dotted line is fitted for subject R.N.'s data using the least squares method ($r = 0.88$). A thick line shows a trend in healthy subjects (derived from Kido et al. 2004).

Fig. 8-1 The TA MEP evoked near the half maximum intensity increased after a single session of FES-aided walking in subject R.T. (young stroke patient). A: MEP input-output curves in the TA muscle are shown before (×), after 20 min (□), and after 30 min of walking (○) with FES (A). Each point shows the average mean rectified MEP (mean ± SE of 4 responses). The increase was most prominent in the middle of the curve (i.e., TMS intensities of 50% and 60%) after 20 min of FES. B, C, and D: MEPs elicited by TMS of 50% maximum output before (B), after 20 min (C), and 30 min (D) of walking with FES are shown. Four responses are superimposed for each measurement. E: Normalized mean rectified MEPs and SPs at TMS of 50% are shown along the experimental elapsed time scale. MEPs measured during 15% MVC of voluntary contraction were normalized by the initial values. Thick horizontal bars from 0 to 35 min show 3×10 min of walking with FES. Circles are for MEPs and squares for SP.

Fig. 8-2 The TA motor output map measured as mrMEP amplitudes before (A) and after (B) one month of regular application of repetitive CP nerve stimulation in subject J.T. (incomplete SCI). Motor output maps were measured during 15% MVC of TA isometric voluntary contraction. After one month of CP nerve stimulation altered the map shape. C: Motor output map obtained by TMS (50% maximum output) in the TA of a healthy female subject (age 27). The largest MEPs were evoked at 1 cm lateral and 0-2 cm posterior to the vertex.

Fig. 8-3 The TA MEP amplitude increased after 6.5 months of FES use in subject M.L. (head injured patient). A and B: Motor output maps were measured during 15% MVC of TA isometric voluntary contraction before (A) and after (B) long-term FES. C: MEPs evoked at TMS of 90% output before and after long-term application of FES. Four responses are superimposed for each measurement. D: TA MEP input-output curves are shown before (○) and after 6 months of FES trial (□). Each point shows the average peak-to-peak MEP (mean ± SE of 4 responses). C and D were measured at the TMS location 3 cm lateral and 1 cm posterior to the vertex.

1. General Introduction

Our somatosensory nervous system activity is continuously modulated, depending on the physical activity and environment surrounding the nervous system. When any specific activity or environment persists, neural connectivity may be modified. This change is often referred to as plasticity. Neural plasticity is the ability of the nervous system to change its structure and function (i.e., connectivity between neurons) in order to adapt to new conditions or environmental demands. This thesis examined modulation and modification of spinal reflexes and corticospinal connections that (1) occur through our daily activities and healthy aging processes, and (2) are induced by external manipulations, such as damage to the central nervous system (CNS) and peripheral nerve stimulation.

1.1 Spinal reflexes

A reflex, an involuntary response to a stimulus applied to the periphery and transmitted to the central nervous system, plays a role in motor control. Excitability of a reflex pathway may be greatly modulated from time to time, task to task, in a manner assisting a goal of the movement to be achieved successfully. Reflex behaviors are the simplest motor behavior or activity. Yet, reflexes are important components of neural networks for motor control, and elucidating how such simple behaviors are modulated in different situations will lead us to a better understanding of more complicated motor control and neural mechanisms behind it. Plasticity in the nervous system, may, therefore, be studied through investigating modulation and modification of reflex pathways.

There are several different spinal reflexes involved in the human motor control, which are induced by sensory stimuli arising from receptors in muscles, joints, or skin.

1.1.1 Reflexes originated from activation of Ia afferents

The **stretch reflex**, a largely monosynaptic reflex from Ia afferents to α-motoneurons, is one of the best known spinal reflexes. When intrafusal muscle fibers are stretched, Ia afferent fibers from the primary muscle spindle endings are activated, and in turn, excite α-motoneurons of the stretched muscle (homonymous excitation). Although Ia afferents also send excitatory inputs to motoneurons innervating other muscles (heteronymous excitation, often to mechanical synergists), usually homonymous excitation is stronger (Eccles et al. 1957a). Excitatory inputs from Ia afferents also have polysynaptic connections to motoneurons. The level of activation of Ia afferents is modulated by activity of γ-motoneurons innervating intrafusal muscle fibers. By preventing spindles from slackening during muscle contraction, γ-motoneurons maintain the responsiveness of muscle spindle endings at different muscle length. In fact, during many voluntary movements α- and γ-motoneurons are more or less coactivated.

The stretch reflex pathway can also be activated by electrically stimulating the peripheral nerve. This electrical analogue of stretch reflex is called **the H- or Hoffmann reflex**. Since the H-reflex is elicited by direct activation of Ia afferents at the axons, it bypasses muscle spindle endings (and the influence of γ-motoneurons).

Monosynaptic components of the stretch and H- reflexes were first suggested by Hoffmann (1918), and later confirmed in cats by Lloyd (1943). Lloyd demonstrated that the afferent discharge evoked by either electrical stimulation of the tibial nerve or the

stretch of calf muscle reached the spinal cord in the fast sensory fibers and made direct contact with motoneurons of the muscle from which the afferents originated. The sum of stretch-evoked afferent response latency recorded at the first sacral (S1) dorsal root and the segmental reflex arc latency recorded at the S1 ventral root in response to the S1 dorsal root stimulation approximated the stretch reflex latency, which only allowed a single synaptic delay. A similar finding was also reported by Renshaw (1940). Later, the monosynaptic component of the H-reflex was also shown in humans by recording the dorsal and ventral cord potentials using long needle electrodes (Magladery and McDougal 1950; Magladery et al. 1951; Teasdall et al. 1952). Although the monosynaptic nature of the stretch or H- reflex was first to be known, oligosynaptic transmission has been demonstrated in cats (Watt et al. 1976; Fetz et al. 1979; Jankowska et al. 1981a; Jankowska et al. 1981b) and humans (Burke et al. 1984).

The stretch reflex arc, especially the H-reflex has been reviewed many times and used extensively in humans for various research purposes (Paillard 1959; Rushworth 1964; Capaday and Stein 1987b; Schieppati 1987; Capaday 1997; Zehr 2002; Misiaszek 2003). The H-reflex is task-dependently modulated in locomotion (Capaday and Stein 1986; Capaday and Stein 1987a; Llewellyn et al. 1990; Edamura et al. 1991; however, see also Simonsen and Dyhre-Poulsen 1999; Ferris et al. 2001). Also, during locomotion the modulation is phase-dependent (Capaday and Stein 1986; Yang and Whelan 1993; Brooke et al. 1995; Lavoie et al. 1997; Schneider et al. 2000; Trimble et al. 2001; Ethier et al. 2003), most likely at least partly supraspinal in origin, rather than due to solely spinal mechanisms.

On activation of Ia afferents, interneurons that have inhibitory connections to motoneurons of the antagonist (Ia inhibitory interneurons) are activated. This process is largely disynaptic, and called **reciprocal inhibition**. Reciprocal inhibition turns off contraction of the antagonist muscles during not only the stretch reflex but also voluntary movements. During movements, extra work against the antagonists is reduced, whereby the action of prime movers is facilitated.

Reciprocal inhibition of motoneurons from activation of antagonist Ia afferents was first experimentally demonstrated by Lloyd (1941; 1946a; 1946b) in a decerebrated cat preparation. A monosynaptic reflex in the gastrocnemius nerve was inhibited by an afferent volley in the deep peroneal nerve. Inhibition was prominent with conditioning-test intervals of 0.5-2 ms, and exponentially decayed over 10-15 ms. Similar observations were made in inhibition of the tibialis anterior by the gastrocnemius afferent volleys. Eccles and his colleagues revealed that inhibition by the quadriceps Ia afferent volleys hyperpolarized postsynaptic biceps-semitendinosus motoneuronal membranes, using intracellular recording in cat motoneurons (Eccles and Lundberg 1958; Eccles 1964). Motoneuronal hyperpolarization was ascribed to generation of the outwardly directed current (i.e., influx of chloride and/or efflux of potassium) at inhibitory synapses.

Human reciprocal inhibition has been studied in two different ways: conditioning of the H-reflex (Mizuno et al. 1971; Tanaka 1974; Crone et al. 1987) and inhibition of ongoing muscle activity (Ashby and Labelle 1977; Ashby and Zilm 1978; Mao et al. 1984; Capaday et al. 1990) by electrical stimulation of the antagonist muscle nerve. Although the original work on reciprocal inhibition was done by conditioning of a monosynaptic reflex (Lloyd 1946a; Lloyd 1946b; Eccles 1964), there are several

drawbacks associated with the H-reflex conditioning in humans. For instance, (1) the amount of inhibition observed on the H-reflex depends on the size of the test reflex (Crone et al. 1990), (2) conditioning of the H-reflex can be performed only in muscles which show sizeable reflexes consistently (Mao et al. 1984), and (3) the H-reflex representing a synchronous discharge of motoneurons in response to large excitatory postsynaptic potentials (PSPs). Inhibitory action by small or moderate size inhibitory PSPs resulting from stimulation of the antagonist Ia afferents may be obscured (Capaday et al. 1990). The other alternative (i.e., measuring inhibition on active motoneurons) can solve these problems with the H-reflex method. Ashby and his colleagues (Ashby and Labelle 1977; Ashby and Zilm 1978; Mao et al. 1984; Ashby and Wiens 1989; Bayoumi and Ashby 1989) investigated the effect of stimulating group I afferents on the tonically active single motoneurons. Inhibition was observed as a decrease of firing probability in a peri- (or post-) stimulus time histogram. Similarly, Capaday et al. (1990) examined inhibition of the tonic muscle activity using the surface EMG, in which the inhibition was measured as a depression in the mean rectified EMG activity. An advantage of surface EMG, compared to recording single motor unit discharges, is that the inhibitory effect can be measured in a population of discharging motor units, and thus, is useful for assessing the amount of inhibition.

The level of activity of Ia inhibitory interneurons is influenced by both spinal and supraspinal inputs. For instance, the efficacy of reciprocal inhibition is different between different types of muscle activation, such as at rest, tonic and dynamic contraction, and co-contraction of agonists and antagonist (Crone et al. 1987; Crone and Nielsen 1989; Nielsen and Kagamihara 1992; Crone and Nielsen 1994). However, task-dependency

and/or function of its modulation has been controversial. This issue is dealt with later in this thesis.

1.1.2 Recurrent inhibition

When α-motoneurons are activated, **Renshaw cells**, another class of inhibitory interneurons, are also activated by recurrent collaterals of α-motoneuronal axons. Recurrent inhibition by Renshaw cells was first studied by Renshaw (1941; 1946). In either decerebrated or anesthetized cats, α-motoneurons of the lumbar cord were monosynaptically excited (Eccles 1931; Eccles and Sherrington 1931) by dorsal root stimulation (i.e., by primary muscle afferents). The activation of motoneurons was conditioned by stimulation of the ventral root, which activated motoneurons antidromically (Renshaw 1941). Antidromic volleys produce small centrifugal discharges from the motoneurons whose axons are stimulated. Renshaw discovered that interneurons receiving inputs from recurrent collaterals send inhibitory inputs to the same (i.e., homonymous) or heteronymous α-motoneurons. Later, it was found that Renshaw cells also inhibit γ-motoneurons (Ellaway 1971), other Renshaw cells (Ryall 1970), and Ia inhibitory interneurons (Hultborn et al. 1971b; Hultborn et al. 1971a). Renshaw inhibition can be investigated in humans by conditioning the test H-reflex with the preceding H-reflex elicited in the same motoneurons. The first (conditioning) reflex discharge is to activate Renshaw cells, and recurrent inhibition can be observed on the second test reflex (Pierrot-Deseilligny and Bussel 1975; Bussel and Pierrot-Deseilligny 1977). Recurrent inhibition is widely distributed in the human lower limb and modulated during voluntary muscle contraction (Katz and Pierrot-Deseilligny 1999).

Renshaw cell action is largely mediated by acetylcholine via nicotinic receptors and L-acetylcarinitine (a cholinomimetic drug) administration potentiates recurrent inhibition (Mazzocchio and Rossi 1997). On the other hand, recurrent inhibition is under a tonic inhibitory influence of serotonergic and noradrenergic inputs. Thus, Renshaw cell excitability might be controlled by reticulospinal projection, where cholinergic and monoaminergic transmission could interact (Mazzocchio and Rossi 1997). Renshaw cells may also contribute to stabilizing motor output (Windhorst and Kokkoroyiannis 1992) and posture maintenance (Katz and Pierrot-Deseilligny 1999).

1.1.3 Ib inhibition

Activation of α-motoneurons and resultant muscle contraction change muscle tension. Golgi tendon organs, located at the junction of muscle fibers and tendon, detect changes in muscle tension and activate Ib afferents. On activation, in general, Ib afferents produce disynaptic and/or oligosynaptic inhibition of α-motoneurons of the contracting muscle (Ib autogenic inhibition). Autogenic inhibition of the contracting muscle was first showed by Granit (1950), who investigated the inhibitory action resulting from generation of muscle tension. Through a series of experiments to test autogenic inhibition, he concluded that inhibition originated from the tension sensitive end organ (i.e., not from primary spindle afferents) and fibers from those "inhibitory" end organs were large, rapidly conducting fibers. Laporte and Lloyd (1952) examined the inhibitory effect of a given muscle nerve stimulation on monosynaptic test reflexes evoked in a synergist muscle; that is, experiments were done in synergist pairs, such as the soleus and gastrocnemius, and the plantaris and flexor digitorum longus. From

differences in threshold and latency between monosynaptic facilitation and observed inhibition, Laporte and Lloyd suggested that the inhibition was mediated by group I afferents via disynaptic pathways. It was also reported that the observed, presumably disynaptic pathways inhibited the homonymous and synergist motoneurons, while facilitating antagonist motoneurons. Eccles et al. (1957b) also studied Ib actions in various cat hindlimb muscles. Their detailed examination showed that in extensor motoneurons, Ib inhibitory connections were frequently observed between motoneurons supplying different muscles, whereas excitatory projections from flexors were rare. On the contrary, flexor motoneurons received Ib excitatory inputs not only from the direct antagonist but also from several different extensors. Moreover, in addition to disynaptic inhibition, trisynaptic linkage was commonly found. In humans, Ib inhibition can be elicited in the soleus muscle by stimulating the nerve to the medial gastrocnemius (MG) muscle; the soleus H-reflex can be significantly reduced by the MG nerve stimulation (Pierrot-Deseilligny et al. 1979).

The action of Ib afferents is not simple, as different excitatory and inhibitory inputs (e.g., from Golgi tendon organs, muscle spindles, cutaneous receptors, and descending pathways) converge onto Ib inhibitory interneurons. Such an extensive convergence of inputs from different sources can even change the direction of response elicited by Ib activation. For instance, at rest cutaneous stimulation of the sole of the big toe depresses Ib inhibitory transmission from the MG muscle to the quadriceps, whereas during voluntary contraction of the triceps, it facilitates Ib inhibition (Pierrot-Deseilligny et al. 1981). More strikingly, Ib afferents from extensor muscles, which generally inhibit activation of extensor muscles, excite extensor motoneurons during locomotion, while

suppressing disynaptic inhibitory pathways (Pearson and Collins 1993; Pearson 1995; see also Stephens and Yang 1996). This is called **reflex reversal**, and is one of the strongest task-dependent reflex modulations known.

1.1.4 Cutaneous reflex

When you accidentally touch a hot stove, you instantly withdraw your hand from the stove. This is called the **flexor withdrawal reflex**, in which the limb is quickly withdrawn from a noxious stimulus. Through polysynaptic pathways the flexors acting at multiple joints are activated while the extensors are inhibited in the stimulated limb. The withdrawal reflex is, thus, a protective reflex. During withdrawal of the limb, another reflex response occurs in the contralateral limb. That is, the contralateral extensors are activated while the antagonistic flexor activity is suppressed via polysynaptic pathways. This **crossed extensor reflex** is to provide postural support at the time of the flexor withdrawal reflex.

Flexor and extensor muscle activities are altered when a nonnoxious stimulus is applied to the cutaneous nerve of the limb (Zehr and Stein 1999b). In cats when tactile stimulation is applied to the foot dorsum during the swing phase of locomotion, the whole limb enhances its flexion to lift the foot over the obstacle. This response is called a stumbling corrective reaction, and the stumbling corrective movements and reflex patterns are phase-dependently modulated during locomotion (Forssberg et al. 1975; Andersson et al. 1978; Forssberg 1979; Duysens et al. 1980). Also, in humans over the step cycle of walking, the sign of cutaneous reflex is completely reversed in the tibialis anterior muscle; the middle latency response changes from excitation during the swing

phase to inhibition during the swing-stance transition (Yang and Stein 1990; Duysens et al. 1992; De Serres et al. 1995). Phase-dependent modulation is also nerve (i.e., stimulus location) specific (Van Wezel et al. 1997; Zehr et al. 1997; Zehr et al. 1998). These findings about cutaneous reflex modulation during locomotion seem to account for roles of cutaneous inputs in stabilization of locomotion against external perturbations. Cutaneous reflexes are also highly task-dependently modulated from standing to walking to running (Duysens et al. 1993; Komiyama et al. 2000).

1.1.5 Presynaptic inhibition

As mentioned above several times, excitability of a reflex pathway is task-, posture-, and/or phase- (during locomotion) dependently modulated, probably by descending inputs, convergence of different inputs from different sources, and **presynaptic inhibition** of the nerve terminal. In cats, presynaptic inhibition was first shown as a decrease of monosynaptic Ia excitatory postsynaptic potentials without any associated changes in the motoneuronal conductance or membrane potential (Frank and Fuortes 1957; Eccles 1964) see also (Gillies et al. 1969). That is, [redacted]ion was not on the postsynaptic neurons, but on the presynaptic terminals at which neurotransmitter release was depressed due to depolarization of presynaptic fibers (i.e., **primary afferent depolarization,** Schmidt and Willis 1963; Eccles 1964). In presynaptic inhibition, reflex transmission is changed by GABAergic interneurons that make axo-axonic synapses near the terminals of afferents. GABA released by interneurons leads to activation of $GABA_A$ receptors in afferent terminals, where efflux of chloride ions results in primary afferent depolarization and thereby reduction of transmitter release (Eccles 1964; Rudomin and

Schmidt 1999). $GABA_B$ receptors also seem to be involved in presynaptic inhibition (Kandel and Siegelbaum 2000; Alford and Grillner 1991). Primary afferent depolarization occurs not only in Ia afferents but also in Ib afferents and cutaneous fibers (Eccles et al. 1963a; Eccles et al. 1963b; Eccles 1964).

In humans several different methods to quantify presynaptic inhibition have been used (Capaday and Stein 1987b; Stein 1995; Capaday 1997; Zehr and Stein 1999a). For example, vibration applied to the tendon or muscle produced inhibition of the H-reflex (De Gail et al. 1966). However, since prolonged vibration can induce several possible effects other than presynaptic inhibition, such as transmitter depletion at Ia terminals and refractoriness of Ia fibers, other techniques have been more widely used for the last few decades. One method is to test presynaptic inhibition in heteronymous Ia projections from the femoral nerve to the soleus motoneurons; the amount of heteronymous Ia facilitation of the soleus H-reflex was used to estimate the amount of presynaptic inhibition on the corresponding Ia afferents (Hultborn et al. 1987a; Faist et al. 1994; Meunier and Pierrot-Deseilligny 1998). That is, a decrease in the facilitation may be attributed to an increase in presynaptic inhibition. Mizuno et al. (1971) tested the effect of common peroneal nerve stimulation on the size of the soleus H-reflex with various stimulus intervals, and reported "D1" inhibition, which appears with 7-20 ms stimulus intervals, likely presynaptic in origin. Similarly, but by stimulation of the common peroneal nerve with longer stimulus intervals (i.e., ≅100ms), presynaptic inhibition of the soleus H-reflex has been tested (Capaday et al. 1995; Zehr and Stein 1999a; Earles et al. 2001). Different methodologies to test presynaptic inhibition show, for example, that presynaptic inhibition of the soleus Ia afferents is greatly modulated during muscle

activation and locomotion (Hultborn et al. 1987b; Meunier and Morin 1989; Nielsen and Kagamihara 1993; Capaday et al. 1995; Faist et al. 1996; Brooke et al. 1997). Presynaptic inhibition of sensory afferents can be mediated by interneurons receiving inputs from different sensory afferents. For instance, inhibition of Ia afferent terminals can originate from Ia afferents of other muscles (Hultborn et al. 1987b; Capaday et al. 1995; Brooke et al. 1997) and cutaneous inputs, as well as corticospinal inputs (Iles 1996). Descending pathways, which also modulate afferent transmissions, are believed to produce presynaptic actions by modulating activity of interneurons mediating presynaptic inhibition.

1.2 Descending motor pathways

In general, reflex excitability is under supraspinal descending control. Here I briefly introduce different descending pathways involved in motor control, locomotion in particular. Most of the information for descending pathways currently available is from a number of studies in quadrupeds (e.g., cats).

1.2.1 Corticospinal tract

The corticospinal tract originates mostly in the motor and premotor areas (Brodmann's areas 4 and 6) and some in somatosensory area (area 3) of the cerebral cortex. Corticospinal neurons, called pyramidal cells, have cell bodies in the layer V of the cortex, and send axons through the internal capsule, cerebral peduncle, and the medullary pyramids. After the medullary pyramids, the majority of neurons decussates in the brainstem and descends to the spinal cord, forming the **lateral corticospinal tract**.

Neurons that do not cross the midline travel down the ventromedial part of the spinal cord to the thoracic levels (**ventral corticospinal tract**). Many neurons in the corticospinal tract have mono- and oligosynaptic connections to the spinal α-motoneurons, as well as connections to Ia inhibitory interneurons, Renshaw cells, and γ-motoneurons. These connections are thought to contribute to fine motor control.

Corticospinal lesions in cats have small effects on simple locomotion (Liddell and Phillips 1944; Laursen and Wiesendanger 1966; Eidelberg and Yu 1981; Armstrong 1986). Usually locomotor function can be regained soon after the placement of lesions, and initial locomotor deficits, such as circumduction, paw drag, and increased extension of the hindlimb (Liddell and Phillips 1944; Eidelberg and Yu 1981; Jiang and Drew 1996; Drew et al. 2002) become much less after two weeks (Armstrong 1986). However, those animals have great difficulties with walking along a narrow beam or a horizontal ladder (Liddell and Phillips 1944). During locomotion, corticospinal neurons are active (Armstrong and Drew 1984) and stimulation of the motor cortex evokes EMG responses in the forelimb muscles (Armstrong and Drew 1985). Drew and his colleagues have clearly demonstrated important contributions of the motor cortex to voluntary gait modifications in cats (Drew 1988; Widajewicz et al. 1994; Jiang and Drew 1996; Drew et al. 2002). In humans, Nathan (1994) reported that after surgical incisions in the spinal cord the extent of motor recovery (or disturbance of motility) was related to the extent of damage in the lateral corticospinal tract. Several recent studies also indicated corticospinal activation of the ankle flexor muscles in human locomotion (Schubert et al. 1997; Capaday et al. 1999; Petersen et al. 2001).

1.2.2 Rubrospinal tract

The rubrospinal tract originates in the red nuclei (* in humans caudal magnocellular part) and crosses the midline near its origin and travels down to the brainstem and the spinal cord. In the spinal cord, rubrospinal neurons make synapses onto interneurons and motoneurons. Neurons in the red nuclei are somatotopically aligned and receive excitatory inputs from the motor cortex and the cerebellum. Rubrospinal neurons generally exert excitatory effects on flexor motoneurons. Orlovsky found that, although destruction of the red nucleus did not cause cessation of locomotion, stimulating the red nucleus increased flexor activities (especially at the knee and ankle joints) during locomotion (Orlovsky 1972c). Also, the activity of rubrospinal neurons was correlated to that of flexor muscles during locomotion (Orlovsky 1972a). Modulation of the rubrospinal activity was abolished after decerebellation and perturbation of movement altered the locomotor activity (Orlovsky 1972a), suggesting influence of cerebellar inputs and ascending spinocerebellar pathways on the rubrospinal tract.

In addition to a general involvement in locomotion (the swing phase, in particular), recently other significant roles of the rubrospinal tract have been indicated. For instance, in cats many forelimb-related rubrospinal neurons increased their activities during voluntary gait modification, peaking in the swing phase (Lavoie and Drew 2002). Interruption of the rubrospinal tract seemed to result in abnormal intralimb coordination of muscle activities in cats walking on a treadmill (Jiang and Drew 1996). A possible role of rubrospinal neurons in skilled locomotion (e.g., a grid walk) has also been suggested in rats (Schucht et al. 2002; Webb and Muir 2003).

1.2.3 Reticulospinal tract

The reticular formation of the brainstem receives inputs from the sensorimotor cortex, the cerebellum, and the spinal cord, and gives off two reticulospinal projections: the **pontine reticulospinal tract** originating in the pons, and the **medullary reticulospinal tract** arising from the medulla. The pontine reticulospinal tract descends mainly ipsilaterally to the spinal interneurons. The medullary reticulospinal tract descends bilaterally, and at the spinal level contributes to inhibition of motoneurons innervating the proximal limbs.

An important involvement of reticulospinal tract in locomotion arises from the projection from the mesencephalic locomotor region (MLR) to the medullary reticular formation (MRF) (Steeves and Jordan 1984; Garcia-Rill and Skinner 1987b). MLR is a well-known locomotor center, as stimulation of MLR induces locomotor activity on a treadmill (Armstrong 1986) and 'fictive' locomotion without afferent feedback (Jordan et al. 1979) in decerebrate cats. MLR stimulation also produces excitatory and inhibitory postsynaptic potentials in spinal motoneurons during locomotion (Shefchyk and Jordan 1985). There have been several studies demonstrating locomotor related activities in MRF, which receives descending inputs from MLR. Drew et al. (1986) found that MRF neurons were active in unrestrained cats walking on a treadmill and discharge patterns in 2/3 of reticulospinal neurons identified were modulated in relation to either recorded EMG activities or the locomotor rhythm. Stimulation of MRF, on the other hand, generated well-organized phasic EMG responses in intact unanesthetized cats (Drew 1991). Cooling of MRF blocked MLR-induced locomotion (Shefchyk et al. 1984), while

electrical or chemical (injection of cholinergic agonist) stimulation of MRF induced locomotor activity (Garcia-Rill and Skinner 1987a). Moreover, destruction of the reticulospinal pathway prevented MLR-induced locomotion in decerebrate cats (Steeves and Jordan 1980). All together, these different observations support the idea that the MLR-MRF-reticulospinal tract connection activates spinal interneurons and thereby generates locomotor activity. In addition to the MLR-MRF-reticulospinal projections, pontomedullary locomotor regions also seem to take part in locomotor activities (Noga et al. 1991).

Recently, other than initiation of locomotion, a few functional implications have been made on the activation of reticulospinal tract during locomotion. Prentice & Drew (2001) showed that reticulospinal neurons were actively involved in gait modification in cats. Furthermore, damage to the reticulospinal tract would result in unstable locomotion and postural deficits (Orlovsky 1972c), although locomotor recovery might be possible (Brustein and Rossignol 1998).

1.2.4 Vestibulospinal tract

There are two vestibulospinal projections, the lateral and medial vestibulospinal tracts. The origin of **the lateral vestibulospinal tract** is in the lateral vestibular nucleus, which receives inputs from the ipsilateral vestibular apparatus (the utricles and saccules). The lateral vestibulospinal tract travels down ipsilaterally in the ventrolateral columns of the spinal cord, serving for postural maintenance and regulation of the extensor muscle tone. The **medial vestibulospinal tract** arises from the medial vestibular nucleus, to which the semicircular canals send signals for the angular acceleration of the head. This

medial tract descends ipsilaterally to the midthoracic levels and plays a role in postural adjustment of the neck and upper limbs. Both vestibulospinal projections produce excitation of extensor and inhibition of flexor motoneurons. Orlovsky (1972c) showed that stimulation of Deiter's nucleus (i.e., lateral vestibular nucleus, the origin of the lateral vestibulospinal tract) facilitated the extensor activity during the stance phase of the step cycle, without influencing the timing of the step cycle, in thalamic and mesencephalic cats. In the similar preparation, the activity of vestibulospinal neurons was phase-modulated during locomotion (Orlovsky 1972b). However, additional decerebellation resulted in disappearance of phase-modulation, as well as reduction of vestibulospinal activity. Together with the effect of limb position on the firing of vestibulospinal neurons, it was suggested that the observed phase-modulation was linked to ascending spinocerebeller tracts and cerebellum outputs. Lesions in the vestibulospinal tract led to wobbly locomotion with poor lateral stability (Brustein and Rossignol 1998), and thus, suggesting the vestibulospinal involvement of locomotor and postural stability.

There are two other descending tracts from the brain to the spinal cord. The tectospinal tract originates in the superior colliculus, decussates in the midbrain, and travels down to the cervical levels. This pathway is thought to mediate orientation of the head and neck in response to visual stimuli. The interstitiospinal tract has its origin in the interstitial nucleus of the midbrain and descends ipsilaterally in the ventromedial part of the spinal cord. Little is known about these two tracts, especially in humans.

For my thesis research, plasticity in the corticospinal tract has been the main interest among those different descending pathways, since the corticospinal tract is the key for (1) locomotor recovery after CNS lesions in humans (Nathan 1994), (2) spinal plasticity, as has been shown in operant conditioning of the stretch and H-reflex in vertebrates (Wolpaw 1997; Wolpaw and Tennissen 2001; Chen et al. 2002; Chen and Wolpaw 2002), and (3) modulation of spinal reflexes as mentioned above.

1.3 Objectives of the thesis

In this thesis, I will present several pieces of evidence for plasticity in the human adult nervous system. The human nervous system activity is continuously modulated and modified at both spinal and supraspinal levels. In healthy human adults, spinal reflexes are modulated during different movements in a short time frame, and in a much longer time frame (i.e., aging process). Descending pathways are also very sensitive to changes in afferent and efferent inputs. When descending pathways are disrupted by trauma to the CNS, both spinal reflexes and descending connections are altered. Is it possible to induce plastic changes in the damaged adult nervous system?

In **chapter 2**, the short-latency, reciprocal inhibitory pathways from the common peroneal (CP) nerve to the soleus muscle and from the tibial nerve to the tibialis anterior (TA) muscle were studied during standing, walking, and running in healthy human subjects. The task- and speed-dependent modulation of spinal reciprocal inhibition was discussed.

In **chapter 3**, excitatory and inhibitory reflexes were examined systematically in healthy human subjects having a wide range of ages. We wished to elucidate how different spinal reflex pathways are affected by healthy aging.

In **chapter 4**, motor-evoked potentials (MEP) by transcranial magnetic stimulation (TMS) in the TA muscle were shown to be facilitated by repetitive electrical stimulation of the CP nerve at intensities above motor threshold. Repetitive stimulation of the CP nerve at 25 Hz for 30 min increased the MEP by 50% at a TMS intensity that initially gave a half-maximum MEP (MEP_h), but not the maximum MEP. Thus, repetitive CP stimulation increased corticospinal excitability, rather than total connectivity.

In **chapter 5**, short-term effects of functional electrical stimulation (FES) of the CP nerve during walking on corticospinal excitability were studied in healthy subjects. This study was a prelude to examining the long-term effects of FES in patient populations, since individuals suffering from foot drop due to CNS lesions have been treated in our laboratory (Wieler et al. 1999). FES was applied to the CP nerve during the swing phase of the step cycle when the ankle flexors are active. Corticospinal excitability was measured in the TA before and after application of FES.

In **chapter 6**, short-term effects of FES-assisted walking on inhibitory and excitatory spinal reflexes were tested. Together with the study of chapter 5, this study was conducted to evaluate short-term effects of FES on spinal and supraspinal pathways in healthy subjects.

In **chapter 7**, spinal reciprocal inhibition of ankle extensor or flexor muscles was investigated in long-standing ambulatory subjects with foot drop. Decreased reciprocal inhibition of the soleus and increased inhibition of the TA were expected, as lesion to the

CNS had caused marked foot drop in those subjects. However, our finding was somewhat surprising. The functional implication of changes in reciprocal inhibition in chronic hemiplegia was discussed.

In **chapter 8**, three different cases of short- or long-term application of FES or FES-like stimulation were reported. These are a part of ongoing FES project for improving hemiplegic gait, and more new cases will be reported in the future. Three patients participated in our study and had foot drop that was treated by FES of the CP nerve. We tried to address the following issues: (1) whether a short-term application of FES increases corticospinal excitability in a patient with CNS lesion and (2) whether the long-term use of FES induces cortical reorganization or alteration of corticospinal connections.

1.4 Studies not included in this thesis

While making progress in the thesis research, I have been involved in several different projects for the last 4-5 years. Here studies not included in the thesis are summarized.

1.4.1 Neural control of rhythmic, cyclical human arm movement: task dependency, nerve specificity, and phase modulation of cutaneous reflexes

Based on the previous findings in reflex modulation in the leg, it was hypothesized that cutaneous reflexes would show task dependency and nerve specificity in the upper limb during rhythmic cyclical arm movement as has been demonstrated in the human lower limb. EMG was recorded from 10 muscles crossing the human shoulder,

elbow and wrist joints while bilateral whole arm rhythmic cyclical movements were performed on a custom-made, hydraulic apparatus. Cutaneous reflexes were evoked with trains (5×1.0 ms pulses at 300 Hz) of electrical stimulation delivered at non-noxious intensities to the superficial radial, median and ulnar nerves innervating the hand. Cutaneous reflexes were phase-dependently modulated, and there was evidence for nerve specificity of reflex modulation during rhythmic movement of the upper limbs. Task-dependent modulation was also seen as cutaneous reflexes were of larger amplitude or inhibitory (reflex reversal) during arm cycling as compared to static contraction. While there are some differences in the patterns of cutaneous reflex modulation seen between the arms and legs, it is concluded that cutaneous reflexes are modulated similarly in the upper and lower limbs implicating similar motor control mechanisms.

This work has been published by Zehr E.P. and Kido A. in J. Physiol. 537: 1033-1045, 2001, and by Zehr, E. P., Carroll, T. J., Chua, R., Collins, D. F., Frigon, A., Haridas, C., Hundza, S., and Thompson, A.K. in Can. J. Physiol. Pharmacol. 82: 556–568, 2004.

1.4.2 Submaximal and maximal cardiorespiratory responses of leg wheeling and arm wheeling in a new wheelchair prototype

This study examined the cardiorespiratory responses to propelling a new wheelchair prototype using a unique type of leg wheeling (LW) that allowed the subjects to propel the wheelchair using bilateral knee extension/flexion. Comparisons were made to arm wheeling (AW) and to treadmill running (TR) using a standard maximal oxygen consumption test to exhaustion. Nine female able-bodied subjects performed 3

incremental submaximal exercise bouts followed by exercise to exhaustion using AW and LW. The results showed that LW was more efficient than AW as evidenced by lower cardiorespiratory effort at the same submaximal intensity during LW, and a lower physiological cost index (PCI - change in heart rate divided by velocity of wheeling) during maximal workloads. LW also elicited significantly higher peak cardiorespiratory values than AW but not TR. The cardiorespiratory variables measured during LW were also highly reliable. In conclusion, this unique form of LW was more efficient than AW during submaximal and maximal exercise.

This work has been published by Tubman, L.A., Bell, G.J., Kido, A., James, K.B., Haykowsky, M.J. and Stein, R.B. in Research in Sports Medicine 12: 115-133, 2004, and by Stein, R.B., Chong, S.L., James, K.B., Kido, A., Bell G.J., Tubman, L.A., & Belanger, M. in Prog. Brain. Res. 137: 27-34, 2002.

1.4.3 Speed and efficiency in walking and wheeling with novel stimulation and bracing systems after spinal cord injury: a case study

The purpose of the study was to compare various systems for locomotion in a single individual with a motor-complete, spinal cord injury. His speed was fastest with a conventional manual wheelchair in which he averaged nearly 2 m/s around a 200 m indoor track in a 4 min. test. His speed was about 30% less, but the Physiological Cost Index (PCI) was lowest (highest efficiency) using functional electrical stimulation (FES) of the quadriceps and hamstring muscles to propel a new, leg-propelled (LegPro) wheelchair. He could walk with knee-ankle-foot orthoses (KAFOs), using a swing-to gait, but his speed was an order of magnitude less (8.8 m/min.) and the PCI an order of

magnitude higher. As a result he only uses these braces on rare occasions. Using a new stance-control KAFO and electrical stimulation of the quadriceps and common peroneal nerve (flexor reflex), he can walk with an alternating gait. The knee flexes during swing and is extended to lock the brace during stance. Once locked, stimulation is turned off and he can rest and catch his breath, if required. His speed has been steadily increasing and the PCI decreasing, but the speed is still slower (4-5 m/min) and the PCI higher than with the conventional KAFOs. Nonetheless, he prefers this method of walking, because of the more normal looking gait and uses it on a daily basis for exercise. Walking with FES and only ankle-foot orthoses (AFOs) yielded the lowest speed (3.5 m/min.) and highest PCI, because of the need to stimulate the quadriceps during the whole stance phase to support the body weight. In conclusion, the LegPro wheelchair provides a more efficient method of locomotion and may help to build up the leg muscles and reduce overuse injuries in the arms. A new stance-controlled KAFO with FES may provide a more acceptable walking system, but must be tested on other subjects.

This work has been submitted for publication by Stein, R.B., Hayday, F., Chong, S.L., Thompson, A.K., Rolf, R., James, K.B., and Bell, G. in Arch. Phys. Med. Rehabil.

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2. SPINAL RECIPROCAL INHIBITION IN HUMAN LOCOMOTION

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2.1 Introduction

Modulation of spinal inhibition has been studied in several motor tasks in humans (Simoyama and Tanaka 1974; Shindo et al. 1984; Crone et al. 1987; Crone and Nielsen 1989; Nielsen and Kagamihara 1992; Nielsen and Kagamihara 1993). In general, reciprocal inhibition is influenced by voluntary activation of antagonist muscles in various postures (e.g., lying, sitting, standing) in healthy humans, likely from a central origin (Nielsen et al. 1992; Nielsen et al. 1995; Kasai et al. 1998; Perez and Field-Fote 2003). The effect of antagonist muscle nerve stimulation on tonically active human single motoneurons was first examined by Ashby and his colleagues (Mao et al. 1984; Ashby and Wiens 1989; Bayoumi and Ashby 1989). Reciprocal inhibition was observed as the period of reduced firing probability in a peristimulus time histogram of the target muscle, which appeared at the latency similar to that of the H-reflex.

2.1.1 Task-dependence

Capaday et al. (1990) studied reciprocal inhibition of ongoing soleus EMG activity induced by the common peroneal (CP) nerve stimulation during tonic voluntary activity and walking in healthy subjects. The amount of inhibition was positively correlated with the background EMG level and stimulus intensity used, but did not differ between the two tasks. Lavoie et al. (1997) examined reciprocal inhibition of the soleus H-reflex in

relation to the TA EMG level in several motor tasks. These authors found an almost linear decrease of the soleus H-reflex amplitude with increasing TA EMG level during TA tonic voluntary contraction and leaning backward (i.e., postural maintenance) while standing. The strength of reciprocal inhibition did not differ between these two tasks.

Petersen et al. (1999) investigated the modulation of disynaptic inhibition by stimulating either the CP or tibial nerve during standing and walking. In their results, the amount of inhibition of on-going EMG activity was larger in standing than in walking for both the soleus and TA muscles. Therefore, they suggested that the reciprocal inhibition between ankle extensors and flexors is task-dependent. They attributed the difference between their observations and those of Capaday et al. (1990) to the different stimulus intensities used to induce reciprocal inhibition. They reported that the task-dependent modulation of inhibition could only be observed when the stimulus intensity was below 1.2 × motor threshold (MT). Petersen and his colleagues also tested reciprocal inhibition of the soleus H-reflex with activation of the Ia inhibitory pathway by CP nerve stimulation. In their study, the soleus H-reflex could be reliably evoked during the swing phase in only 4 out of more than 30 subjects tested. In those 4 subjects, when the size of the test reflex was kept constant at 5% of the maximum M-wave (M_{max}) throughout the step cycle, the H-reflex amplitude was significantly decreased by the CP nerve stimulation only in the swing phase. Thus, they concluded that disynaptic Ia reciprocal inhibition between ankle flexors and extensors is modulated during walking. To resolve these discrepancies we wished to study reciprocal inhibition under a wider range of tasks, as has been used for the H-reflex. The phase- and task-dependent modulation of the H-reflex is a well known phenomenon (Capaday and Stein 1986; Capaday and Stein 1987;

Edamura et al. 1991; Yang and Whelan 1993; Brooke et al. 1995; Garrett et al. 1999; Schneider et al. 2000; Trimble et al. 2001; Ethier et al. 2003). The modulation from standing to walking, to running is generally clear and strong (Capaday and Stein 1986; Capaday and Stein 1987; Edamura et al. 1991, but see also Simonsen and Dyhre-Poulsen 1999; Ferris et al. 2001; Dyhre-Poulsen and Simonsen 2002; Simonsen et al. 2002).

2.1.2 Methodological issues

In contrast, the task-dependent modulation of reciprocal inhibition has not been studied to a similar extent. Some of the reasons are methodological. Although many studies have analyzed inhibition of the H-reflex (Mizuno et al. 1971; Simoyama and Tanaka 1974; Tanaka 1974; Shindo et al. 1984; Crone et al. 1987; Crone and Nielsen 1989; Nielsen and Kagamihara 1992; Nielsen and Kagamihara 1993; Capaday et al. 1995; Lavoie et al. 1997; Kasai et al. 1998; Perez and Field-Fote 2003), H-reflex inhibition is problematic during walking. The H-reflex changes greatly during the step cycle and often is not seen at all during some phases (Capaday and Stein 1986; Lavoie et al. 1997; Schneider et al. 2000). Inhibition and excitation are also very dependent on the size of the H-reflex (Crone et al. 1990). Also, it might be difficult to ensure that the tibial nerve stimulus was unchanged, as well the common peroneal stimulus throughout the dynamic movement (Capaday 1997). Therefore, in the present study, inhibition was measured as a decrease in the ongoing EMG activity, not as a decrease in the H-reflex.

Modulation of reciprocal inhibition was studied in relation to three different tasks (i.e., standing, walking, and running) at several different speeds in healthy adults. The question asked was whether reciprocal inhibition is modulated between different tasks.

First, the effect of stimulus intensity on the amount of inhibition was examined to determine a proper stimulation for studying a task-dependence. In other words, we reexamined whether a weaker stimulation (i.e., 1.1 × MT) is better to observe a task-dependence, as argued by Petersen et al. (1999). Then, for the first time, reciprocal inhibition was investigated during running, and compared with the inhibition during standing and walking. We found that the inhibition decreased substantially with increasing speed, but showed a task-dependence, which was only significant in some comparisons.

2.2 Materials and methods

2.2.1 General procedure

Sixteen neurologically normal subjects aged from 25 to 61 participated in this study. All subjects gave informed consent for the purposes and procedures of the experiments, as approved by the Human Ethics Committee of the University of Alberta. Electromyography (EMG) was recorded from the tibialis anterior (TA) and soleus muscles with surface self-adhesive Ag-AgCl electrodes (Kendall LTP, Chicopee, MA). At the beginning of each experiment, the maximum tonic voluntary contraction (MVC) of each muscle was determined as the maximum rectified EMG level. During standing, the subject was asked to voluntarily control a target muscle EMG level for either plantar flexion or dorsiflexion to match a preset level on an oscilloscope (see the section *EMG recordings and electrical stimulation*). Then, reciprocal inhibition of the ankle extensors and flexors was elicited by stimulating the common peroneal (CP) or the tibial nerve respectively, while the subject was standing upright, walking or running on a treadmill.

For 14 subjects, reciprocal inhibition of the soleus EMG activity was examined by CP nerve stimulation. First, the effect of stimulus intensity on the amount of inhibition in the soleus EMG activity was investigated. Under fixed tonic contraction (about 15% MVC) of the soleus, the CP nerve stimulus intensity was varied from the TA threshold to the maximum M-wave. Four trials were recorded at each stimulus intensity. Collected data were immediately analyzed by a custom-written program in MATLAB (Mathworks Inc., Natick, MA) to determine the motor threshold (MT) of CP nerve stimulation for further experimental procedures. Then, the effect of soleus contraction level on the amount of inhibition was tested at a variety of soleus tonic contraction levels using 1.5 × MT of the CP nerve stimulation, which produced clear inhibition in all subjects. Similarly, inhibition of soleus EMG activity elicited by the CP nerve stimulation was studied during walking. Ten of 14 subjects were asked to walk on a treadmill at a comfortable speed (≅ 4 km/h) for 4 min, while the CP nerve stimulation (1.5 × MT) was applied at all parts of the step cycle with random intervals (1.6 to 2.7 s). The same protocols were used for the tibial nerve stimulation and the inhibition of TA voluntary activity. For 7 subjects, a stimulus intensity of 1.1 × MT was also used for both the CP and tibial nerve stimulation to examine the effect of stimulus intensity on the task-dependent modulation of reciprocal inhibition. From the comparison of results between two stimulus intensities, (see Results and Fig. 2-3), 1.5 × MT of stimulation was chosen for the rest of the study. Subsequently, in 8 subjects inhibition of the ongoing EMG activity was investigated by stimulating either the CP nerve or the tibial nerve during standing, walking at either 3 or 6 km/h, and jogging or running at either 6 or 9 km/h.

Amplitudes of M-waves were monitored throughout each experiment, to maintain a steady level of stimulation.

2.2.2 EMG recordings and electrical stimulation

The soleus and TA EMG signals were obtained using surface self-adhesive Ag-AgCl electrodes (Kendall LTP, Chicopee, MA). EMG recording electrodes were placed 2-3 cm below the gastrocnemius in line with the Achilles tendon for the soleus, and over the motor point for the TA, with ~2 cm interelectrode spacing. The signals were amplified, high-pass filtered at 10 Hz, low-pass filtered at 1 kHz, and recorded with Axoscope (Axon Instrument, Union City, CA) at a sample rate of 5 kHz. EMG signals were also rectified, low-pass filtered at 3 Hz, and sent to an oscilloscope, so that subjects could monitor their EMG activity levels during experiments. During standing EMG and nerve stimulus signals were recorded for 300 ms, including a prestimulus period of 40 ms, in response to each test stimulus pulse. During walking heel contact was detected by a force-sensitive resistor (Interlink, Camarillo, CA) inserted between a subject's shoe and foot. Heel contact was used to define the beginning of the step cycle.

The electrical stimulation was applied to the CP nerve and/or the tibial nerve by surface Ag-AgCl electrodes (Kendall LTP, Chicopee, MA). The stimulus electrode for the CP nerve was placed at a low threshold point near the neck of the fibula where activation of TA was prominent and activation of peroneal muscles was weak, and the anode electrode was located below the patella. The electrodes for the tibial nerve stimulation were placed in the popliteal fossa and over the patella. Stimulus pulses were 1 ms rectangular pulses and delivered from Grass SD9 isolated stimulators (Grass Inst.,

Quincy MA). The test stimulation was triggered by a random pulse generator, so that the stimuli were generated with pseudo-random intervals (1.6 to 2.7 s).

2.2.3 Data analysis

To evaluate the effect of antagonist muscle nerve stimulation on the target muscle activity, the target muscle EMG was rectified, and the mean level of EMG amplitude was measured for a 7-10 ms period including the peak depression (Fig. 2-1). When the period of inhibition appeared to be longer than 20 ms, the first 10 ms was used for analyses. Since it is measured over a period of time, the inhibition may contain disynaptic and other short latency reflex components. We will refer to this as short latency reciprocal inhibition or simply reciprocal inhibition, without specifying neural pathways that may be involved. To measure the background EMG activity level for standing, a 40 ms prestimulus period was used. For walking and running, the average EMG activity was computed for unstimulated steps (>50 steps), and used as the control EMG (Capaday et al. 1990; Yang and Stein 1990; Petersen et al. 1999). The amount of reciprocal inhibition was expressed as the difference in the mean rectified EMG level in the chosen 7-10 ms period from the background studied in 40 ms prior to stimulation or in the control EMG of unstimulated steps. On calculating the amount of inhibition, some of the collected responses evoked by too strong or too weak stimuli (indicated by amplitudes of M-waves) were eliminated from the further analysis.

In order to observe the extent of inhibition at different times in the step cycle, inhibition during walking or running was sorted into different bins according to the time at which the stimulation was given. For each subject the step cycle was divided into 16

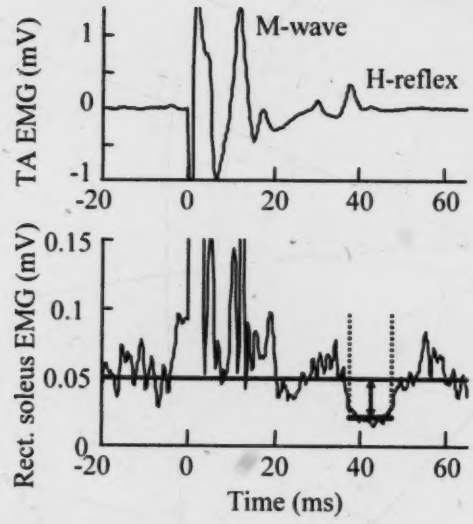

Fig. 2-1 Stimulating an antagonist muscle nerve decreases ongoing voluntary EMG activity. Reciprocal inhibition of the soleus EMG activity was observed following the H-reflex in the TA by stimulation at 1.5 × MT of the common peroneal (CP) nerve. Note that the positive signal of 0-20 ms in the rectified soleus EMG is pickup from activation of the CP nerve innervated muscles (e.g., extensor digitorum longus and peroneus longus) The mean level of inhibition (mV) was measured for a 7-10 ms window (see short bar) including the peak depression in the rectified EMG. (The amount of inhibition is indicated as an arrow.) The significance of inhibition was assessed by either the ratio or difference between the mean inhibition level and the mean rectified background EMG level. Background EMG levels were calculated from 40 ms of prestimulus period for standing data and from the corresponding 7-10 ms window of unstimulated steps for walking data. The long horizontal line indicates the background EMG level. The time 0 refers to the time of nerve stimulation. Twenty sweeps were averaged for each part of the figure.

equal bins, and the inhibitory responses produced by stimuli within the same bin were averaged together (Capaday and Stein 1986; Capaday and Stein 1987; Edamura et al. 1991).

2.3 Results

2.3.1 Effects of stimulus intensity on inhibition of ongoing EMG activity

When the CP nerve stimulus intensity increased (Fig. 2-2A), the amount of inhibition in the soleus EMG increased (for calculation of inhibition, see *Materials and methods*). Although the TA H-reflex could be evoked in some subjects as in Fig. 2-1, the amplitude was often small. Even in subjects without clear H-reflexes, inhibition of the ongoing soleus EMG was observed. Thus, the presence or absence of the H-reflex seemed not to affect the amount of inhibition in the soleus EMG. Inhibition of the soleus EMG as a fraction of background EMG in all subjects is shown against the stimulus intensity expressed as a ratio to the TA motor threshold (MT) in Fig. 2-2A. An exponential curve fitted by least mean squares method had a correlation coefficient of 0.60, and indicated the threshold for inhibition of 0.80. Thus, the fibers responsible for the inhibition have a threshold lower than the M-wave threshold and are presumably primary muscle spindle afferents (Mao et al. 1984; Ashby and Wiens 1989; Capaday et al. 1990; Petersen et al. 1999). However, near the threshold, some subjects showed no inhibition, while some others showed some inhibition. Clear inhibition was observed in all subjects with stimulus intensities of $\geq 1.4 \times$ MT.

The effect of tibial nerve stimulus intensity on the amount of inhibition in the TA was investigated in all 14 subjects. Inhibition of the TA normalized by the background

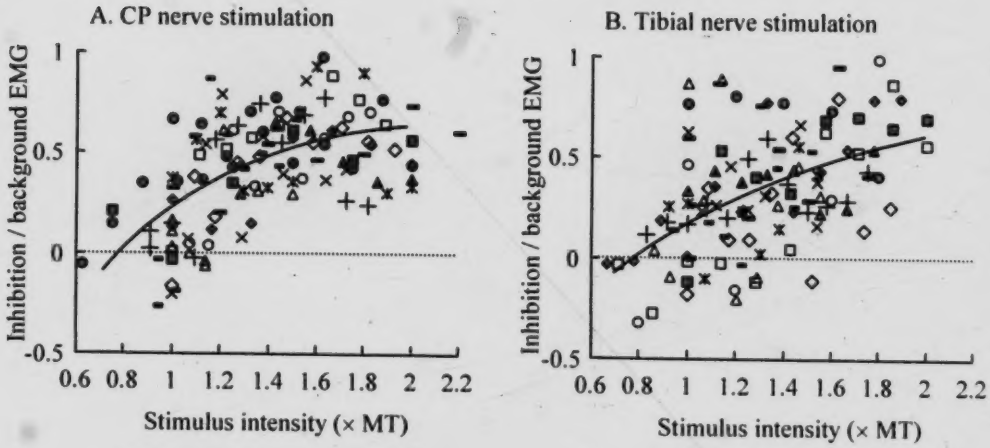

Fig. 2-2 Reciprocal inhibition with different intensities of antagonist muscle nerve stimulation. A: Inhibition of the soleus EMG as a fraction of background EMG in all subjects was plotted against the stimulus intensity expressed as × MT. Each symbol represents a single subject. An exponential curve was fitted to the group data (r=0.60). B: Inhibition of the TA EMG as a fraction of background EMG in all subjects was plotted in relation to the stimulus intensity expressed as × MT. Correlation coefficient of the fitted exponential curve was 0.49.

EMG is plotted in relation to the tibial nerve stimulus intensity in Fig. 2-2B. A fitted exponential curve (r=0.49) indicated the threshold for inhibition of 0.79, which is again below the M-wave threshold. Compared to the soleus, the stimulation-inhibition relation was less clear in the TA, although most subjects showed inhibition with the stimulation above 1.4 × MT. Thus, a stimulus intensity of 1.5 × MT was mainly used in this study for both the soleus and TA, because it reliably produced inhibition of ongoing EMG activity.

In Fig. 2-3 reciprocal inhibition in the soleus EMG during standing and walking at 4 km/h is plotted against different background EMG levels at 1.5 (A) and 1.1 (B) × MT of stimulation. As seen in Fig. 2-3A, the higher the soleus EMG background level, the larger the amount of inhibition with a stimulus intensity of 1.5 × MT. A dashed line is drawn which corresponds to 100% inhibition. The means ± SD of the correlation coefficients (Pearson's *r*) for all subjects were 0.90±0.08 for standing and 0.76±0.17 for walking. Group means of regression slopes were significantly greater than 0 for both standing (0.54±0.13, $P<0.0001$; Student's one sample t-test) and walking (0.34±0.12, $P<0.0001$), indicating significant effects of background EMG level on the amount of inhibition, and a significant difference between the tasks ($P<0.01$; Student's paired t-test). For a fair comparison between the tasks, regression lines were also calculated for the same background EMG range (from 13.1±1.8 to 70.0±5.8% MVC). The group mean ± SD of slopes for standing was 0.49±0.15, and was significantly larger ($P<0.05$; Student's paired t-test) than that for walking (0.38±0.12).

The reciprocal inhibition was also examined in 7 subjects, using a stimulus intensity of 1.1 × MT. Fig. 2-3B is an example of the soleus inhibition induced by 1.1 ×

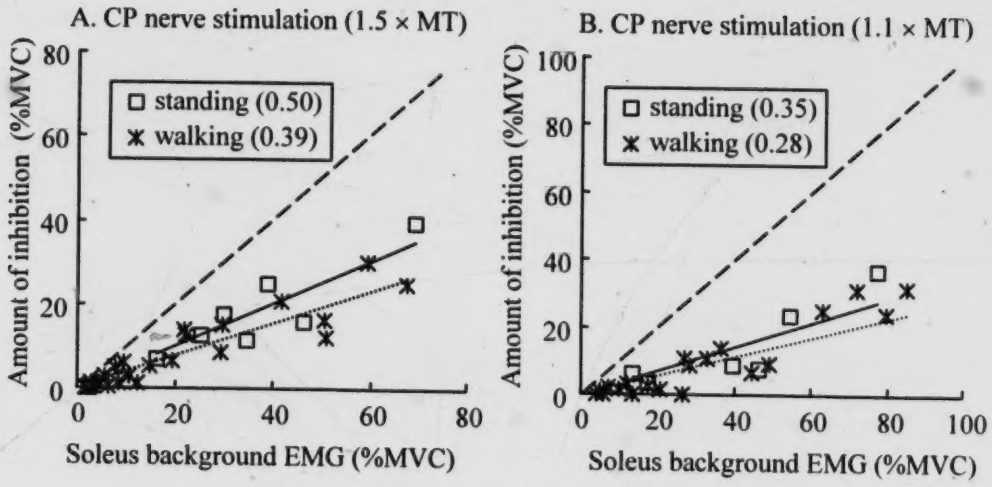

Fig. 2-3 Inhibition of ongoing EMG activity increases as the background EMG activity increases. The amount of inhibition as the difference between the mean rectified EMG of a selected 10 ms window and the background (BG) EMG is presented as a function of BG EMG levels for standing and walking. To show the level of muscle activation, both the inhibition and BG EMG are expressed as % MVC. Stimulus intensities of 1.5 × MT (A) and 1.1 × MT (B) were used to examine the effect of stimulus intensity on inhibition of ongoing EMG activity. Dashed lines are unity lines indicating complete suppression of ongoing EMG activity. Each point is the average of 4 responses that are the closest in the background EMG for standing or in the step cycle for walking. Values given in the figures indicate slopes of regression lines (standing: solid, walking: dotted). Both A and B are from subject AK.

MT of the CP nerve stimulation in a single subject. In this subject, the amount of inhibition increased with an increase of the background EMG level in both standing and walking; slopes of regression lines were 0.35 for standing and 0.28 for walking (not significantly different). The group means ± SD were 0.36±0.12 for standing and 0.26±0.12 for walking, and there was no significant difference between the tasks (P=0.06). When slopes of regression were compared for the same range of background EMG activity (from 12.3±1.1 to 69.4±6.3 %MVC), the means ± SD were 0.30±0.09 for standing and 0.25±0.07 for walking, again showing no significant difference (P=0.10). The amount of inhibition indicated by the slope was larger with 1.5 × MT than with 1.1 × MT in standing (P<0.001) but not in walking (P=0.30).

Similarly, for the soleus, the amount of reciprocal inhibition in the TA was examined with different background EMG levels at stimulus intensities of 1.5 and 1.1 × MT. The correlation coefficients were 0.73±0.22 for standing and 0.63±0.35 for walking at 4 km/h. Slopes of regression lines were significantly greater than 0 for both standing (0.45±0.24, P<0.0001) and walking (0.43±0.28, P<0.0001), and not different between the tasks (P=0.68). When regression lines were calculated for the same background EMG range (from 8.2±0.9 to 33.4±2.9 %MVC) for a fairer comparison, the group means ± SD of slopes were 0.40±0.24 for standing and 0.44±0.28 for walking, and not different between the two tasks (P=0.41). Inhibition of the TA EMG was also examined with a stimulus intensity of 1.1 × MT. When regression lines were compared between the tasks, slopes were not significantly different (standing: 0.39±0.18, walking: 0.24±0.10, P=0.10). When the slope comparison was made for the same background EMG range (from 6.5±2.0 to 29.3±4.7 %MVC), the difference was less: (standing: 0.34±0.22, walking:

0.24±0.09, P=0.37). Thus, the task-dependent modulation of reciprocal inhibition was not demonstrated with stimulation of the tibial nerve at either 1.5 or 1.1 × MT. Different from the observation for inhibition of the soleus EMG, the slope of inhibition was not significantly different between 1.5 and 1.1 × MT in either standing (P=0.09) or walking (P=0.08).

Overall, the same trends were seen with 1.1 × MT as for 1.5 × MT, but the amount of inhibition was larger for 1.5 × MT and the differences between conditions were statistically significant. Therefore, we used 1.5 × MT to investigate task-dependent modulation of reciprocal inhibition.

2.3.2 Inhibition of ongoing EMG activity during walking and running

Inhibition of the ongoing EMG activity was investigated in 8 subjects by stimulating either the CP nerve or the tibial nerve using a stimulus intensity of 1.5 × MT during standing, walking at either 3 or 6 km/h, and jogging or running at either 6 or 9 km/h. The means ± SD of the step cycle duration were 1261±112 ms for slow walking (3 km/h), 984±48 ms for fast walking (6 km/h), 801±42 ms for slow running (6 km/h), and 762±33 ms for running (9 km/h).

The time course of change in the soleus inhibition for each locomotor condition is shown in Fig. 2-4A-D. The step cycle was divided into 16 equal bins, and inhibitory responses produced by stimuli within the same bin were averaged together for each subject. Approximately 10 responses were contained in each bin. The mean rectified EMG (i.e., background EMG level) calculated in unstimulated steps and the anticipated amount of inhibition were also averaged for each bin, and plotted with the actual measure

of inhibition. The *anticipated* inhibition was calculated from the standing condition for a given level of background EMG activity, and reasonably reliable, as the correlation coefficient of linear regression between the background EMG level and the amount of inhibition was high for standing (r=0.88±0.04, mean ± SE).

As can be seen in Fig. 2-4A-D, the actual amount of inhibition never exceeded the anticipated inhibition, although the difference between the anticipated and actual inhibition changed, depending on the background EMG level and locomotor condition. The difference was small for slow walking, whereas it was large for running. Results of one-way repeated ANOVA performed on the inhibition in 16 bins revealed significant effects of time course in the step cycle on the amount of inhibition for all 4 locomotor conditions (P<0.0001). However, when the inhibition was expressed as a fraction of the background activity level, no single bins were significantly different from others.

Similarly, the time course of the TA inhibition is presented in Fig. 2-4E-H, together with the amount of inhibition estimated from standing condition and the background EMG activity. A high correlation coefficient of linear regression between the background activity and the amount of inhibition (r=0.96±0.01) for standing likely gave reliable estimates of inhibition to a given background EMG level. The amount of inhibition actually measured was always smaller than the anticipated amount, indicating the difference in reciprocal inhibition between standing and walking or running. Significant effects of time course on the inhibition were detected by one-way repeated ANOVA performed on 16 bins for all 4 conditions (P<0.0001). When the amount of inhibition was normalized by the background activity, however, there was no difference among bins.

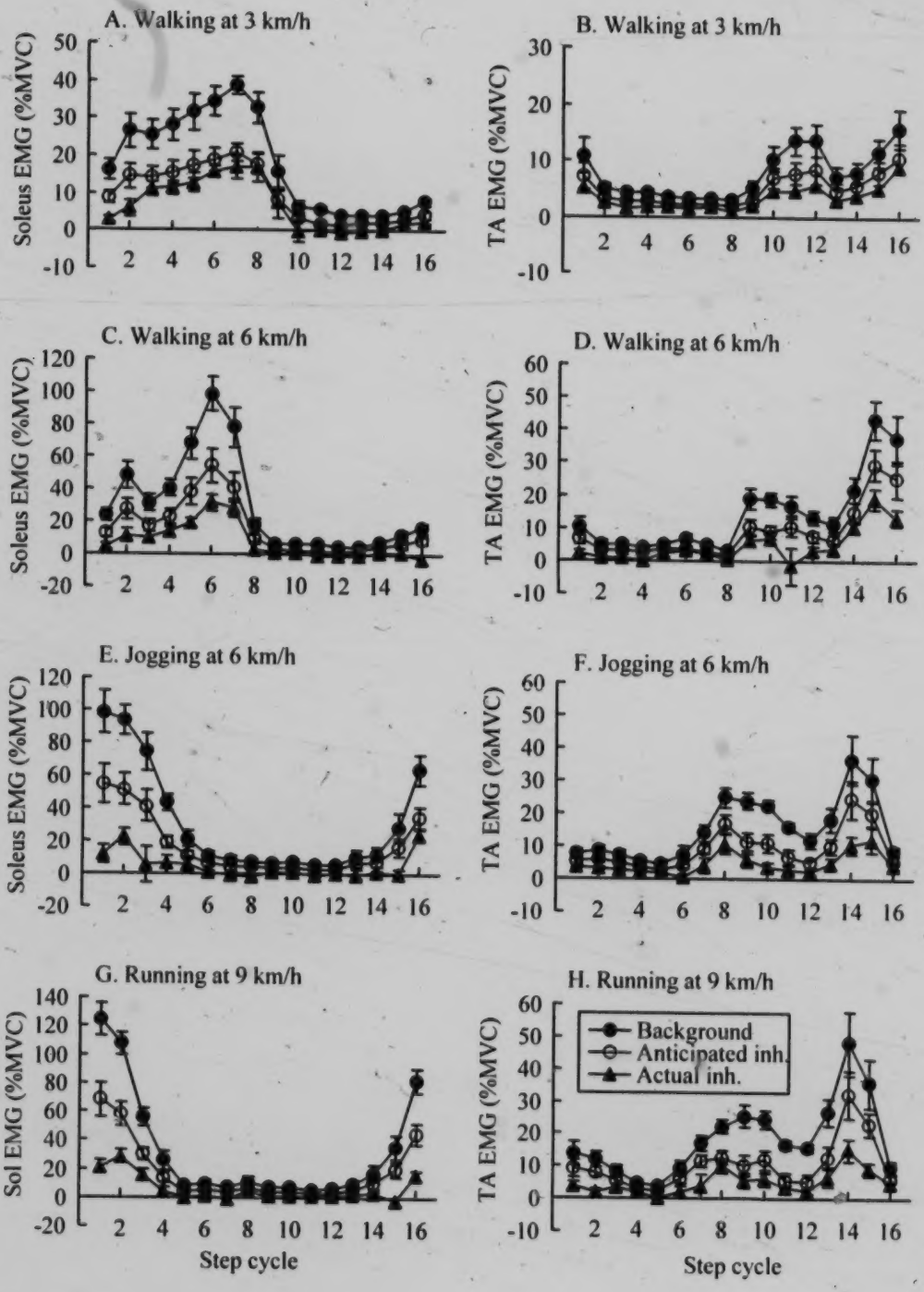
A. Walking at 3 km/h
Soleus EMG (%MVC)
B. Walking at 3 km/h
TA EMG (%MVC)
C. Walking at 6 km/h
Soleus EMG (%MVC)
D. Walking at 6 km/h
TA EMG (%MVC)
E. Jogging at 6 km/h
Soleus EMG (%MVC)
F. Jogging at 6 km/h
TA EMG (%MVC)
G. Running at 9 km/h
Sol EMG (%MVC)
H. Running at 9 km/h
TA EMG (%MVC)
Background
Anticipated inh.
Actual inh.
Step cycle
Step cycle

Fig. 2-4 The time course of change in the inhibition is shown with the background EMG activity for each locomotor condition. The step cycle was divided into 16 equal bins, and inhibitory responses produced by stimuli within the same bin were averaged together for each subject. The mean rectified EMG (i.e., background EMG level) calculated in unstimulated steps and the amount of inhibition anticipated from the standing condition were also averaged for each bin for each subject. Filled circles are for group means of background EMG levels, open circles for group means of anticipated inhibition, and triangles for group means of observed inhibition. Vertical bars indicate ± SE. Inhibition of the soleus EMG is presented in A, C, E, and G, and inhibition of the TA EMG is presented in B, D, F, and H. A and B: Slow walking (at 3 km/h), C and D: fast walking (at 6 km/h), E and F: slow running (i.e., jogging at 6 km/h), and G and H: running (at 9 km/h).

Inhibition of the ongoing soleus EMG activity was expressed as a function of background EMG activity for 5 different conditions. Group means of regression slopes, calculated for the full range of background EMG activity, are presented in Fig. 2-5A. With increasing speed (from standing, walking, to running), the slope becomes less steep and the correlation becomes weaker. The result of one-way repeated ANOVA showed a significant effect of condition on the slope (F=16.6, P<0.0001). Slopes were also compared between two conditions using Student's paired t-test. Significant differences between the conditions are indicated by * (P<0.05) or ** (P<0.01) in Fig. 2-5A. Note that some of the differences might appear to be significant by chance since 10 paired comparisons were made between tasks. However, most conditions were highly significantly different. For example, the slope for standing (mean ± SE: 0.515±0.050) was steeper than those for walking at 6 km/h (0.305±0.043), running at 6 km/h (0.199±0.040), and running at 9 km/h (0.269±0.040), but not for walking at 3 km/h (0.411±0.062). No significant difference was seen between running at 6 km/h and 9 km/h. The mean ± SE of the correlation coefficients between the background EMG and the amount of inhibition was 0.87±0.04 for standing, 0.75±0.07 for walking at 3 km/h, 0.75±0.05 for walking at 6 km/h, 0.55±0.09 for running at 6 km/h, and 0.52±0.08 for running at 9 km/h. Although the correlation became less when condition changed from standing to walking, to running, the correlations were all statistically significant.

The correlation between inhibition of the ongoing TA EMG activity and the background EMG activity was evaluated for each condition. Group means of regression slopes are presented in Fig. 2-5B. The result of ANOVA revealed a significant effect of condition on the steepness of regression slope (F=16.1, P<0.0001). The mean ± SE of

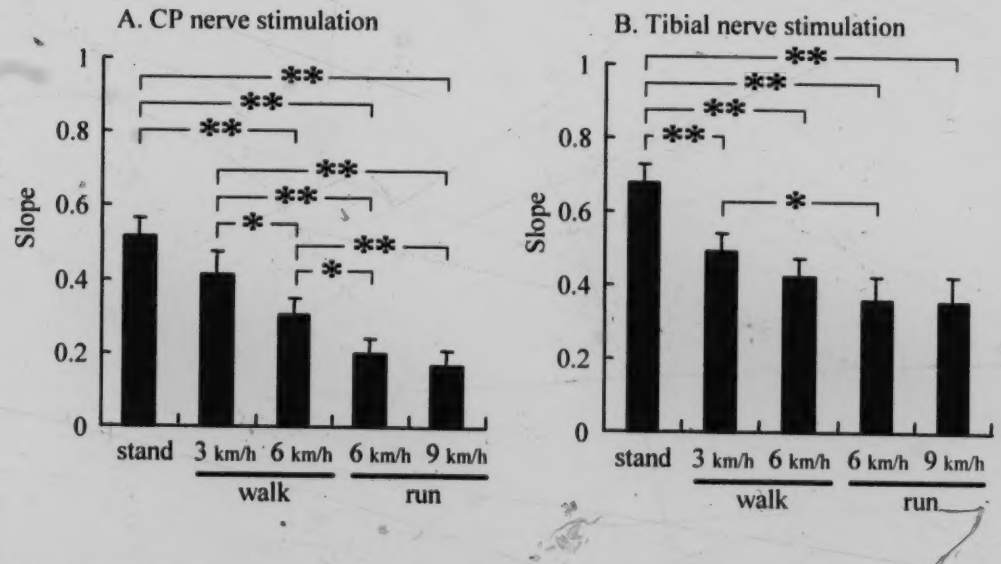

Fig. 2-5 The amount of inhibition decreases from standing to walking, to running. A: Group means of regression slopes between the background EMG and the amount of soleus inhibition are presented with standard error bars. With increasing speed, the slope becomes less and correlation becomes weaker. B: Group means of slopes for the TA inhibition are presented with standard error bars. A stimulus intensity of 1.5 × MT was used for all conditions. A significant difference between two conditions tested by Student's paired *t*-test is indicated by * ($P<0.05$) or ** ($P<0.01$).

individual slopes for standing was 0.678±0.047, and was significantly steeper than those for walking at 3 km/h (0.492±0.047) and 6 km/h (0.423±0.049), and running at 6 km/h (0.360±0.064) and 9 km/h (0.354±0.068). The difference between walking at 3 km/h and running at 6 km/h ($P<0.05$) was also significant. The correlation coefficient between the background EMG and the amount of inhibition decreased from standing, walking, to running: mean ± SE, 0.96±0.01 for standing, 0.73±0.08 for walking at 3 km/h, 0.75±0.05 for walking at 6 km/h, 0.67±0.07 for running at 6 km/h, and 0.63±0.07 for running at 9 km/h. All of these values were statistically significant.

2.4 Discussion

The main goal of this study was to examine spinal inhibition induced by the antagonist muscle nerve stimulation in relation to a task-dependence. The effect of stimulus intensity on the amount of inhibition was examined to choose a proper stimulation to study the task-dependent modulation of inhibition.

2.4.1 Effect of stimulus intensity on the amount of inhibition

The depression in the rectified EMG increased significantly with the level of activity in both the soleus and TA muscles for our group of subjects with stimulation of the CP and tibial nerves respectively (Fig. 2-3), in agreement with previous work (Capaday et al. 1990; Petersen et al. 1999). The slope relating the inhibition and the background EMG activity with CP stimulation at 1.5 × MT was significantly greater during standing than walking at 4 km/h (54% compared to 34%, 11 subjects), but not at 1.1 × MT (36% compared to 26% in 7 subjects). These results are opposite to those of

Petersen et al. (1999) who found a significant difference at 1.1 × MT (14 subjects), but not at 1.6 × MT (4 subjects). The reason for the discrepancy between the studies is not known. We concentrated on the higher stimulus level since the amount of inhibition increased with stimulus intensity and could then be observed in all subjects (Fig. 2-2). With stimuli close to motor threshold little inhibition was observed in some subjects, so the ability to see differences without averaging very large numbers of stimuli was severely compromised. When the tibial nerve was stimulated, no significant difference in inhibition was observed in the TA muscle between standing and walking with either 1.1 or 1.5 × MT. Petersen et al. (1999) did not report any data on this type of stimulation. A stimulus intensity of 1.5 × MT was, thus, used to study reciprocal inhibition in several different types of movement.

Recently, the axonal excitability of motoneurons has been shown to be activity-dependent (Vagg et al. 1998; Burke et al. 2001; Krawitz et al. 2001; Kuwabara et al. 2001; Kuwabara et al. 2002). Also it has been reported that the maximum M-wave changes during walking and running (Simonsen et al. 1995; Simonsen and Dyhre-Poulsen 1999) or during a long-lasting experiment (Crone et al. 1999). Thus, there was a possibility that constant stimuli that produce the same amplitude of M-waves might not be activating the same population of neurons between and within dynamic motor tasks. However, recently Ferris et al. (2001) showed that the maximum M-wave amplitude changed only up to 20-30% during walking and running Also, as shown in Fig. 2-2A, the amount of inhibition reached a plateau with stimulus intensities ≥ 1.4 × MT. Finally, maintaining the size of M-wave was the best we could do to keep the stimulus intensity constant. We are confident that enough afferents were recruited to activate inhibitory

interneurons throughout an experiment.

2.4.2 EMG activity and correlation coefficient

The most important finding in this study was that the amount of inhibition, indicated by a slope of linear regression between the background EMG and the amount of inhibition, decreased from standing to walking, to running (Fig. 2-5). However, while the slope of regression changed from standing to walking to running, the correlation coefficient also became smaller from standing to locomotion. Perhaps, the reduced slope of inhibition could be due to increased variation in the EMG activity. In fact, the EMG activity level changes more rapidly with increasing speed of locomotion, and that will likely increase the variability of EMG activity and reduce the correlation coefficient. However, Pearson's *r* remained in a statistically significant range over different conditions. Therefore, the calculated slopes of regression lines were still reliable, and a change in the regression slope among different conditions cannot be explained fully by the variability of EMG.

In the present study, the range of background EMG level varied from one condition to another. For instance, the soleus EMG range during running at 9 km/h was three times larger than the range during walking at 3 km/h. So one might ask whether the difference in the range of background EMG activity among different conditions influenced the observed task-dependent modulation of inhibition. However, a slope change (i.e., a change in the amount of inhibition) seemed less likely to be a simple function of background EMG level, as a similar task-dependent modulation was observed in both the TA inhibition, in which the background EMG range was standing > running >

walking, and in the soleus inhibition, in which the EMG range was standing ≤ walking < running.

2.4.3 Spinal inhibition and phase of the step cycle

Previous work has found a task-dependence for inhibiting the soleus H-reflex in the swing phase of walking, compared to standing, by measuring the effect of voluntary TA activity (Lavoie et al. 1997) or of CP nerve stimulation (Petersen et al. 1999) in a few subjects (4 of 30) who showed an H-reflex during the swing phase of walking. We have not repeated those experiments, but compared the amount of inhibition in equally separated 16 bins for 4 different locomotor conditions. Although significant effects of time course in the step cycle were found for all 4 locomotor conditions with the CP nerve stimulation, when the inhibition was normalized by the background activity, no single bins showed significantly more or less inhibition than other bins in any conditions (Fig. 2-4). Inhibition of the TA was also compared among 16 bins separated according to the time course of step cycle. Since the early to mid swing phase (i.e., first burst) and the late swing to transition phase (i.e., second burst) of the TA activity have functionally different roles in human walking and are differently modulated in response to cutaneous inputs (Duysens et al. 1990; Yang and Stein 1990; Duysens et al. 1992; De Serres et al. 1995; Hauglustaine et al. 2001), inhibition of these two bursts might be modulated differently. However, the relative amount of inhibition to the background activity was not significantly different over the step cycle. Thus, the inhibition did not depend on the part of the stance phase for the soleus or the part of the swing phase for the TA. In other words, these results assured us that the data for the whole step cycle could be pooled,

since the inhibition depended mostly on the background EMG level and not on the phase in the cycle.

2.4.4 Task-dependence of reciprocal inhibition

In this study we compared the task dependence of spinal reciprocal inhibition at a short latency with different stimulus intensities, stimulation of different nerves, different analytic procedures and a range of tasks from standing to walking to running. The results are summarized in Table 2-1. Although there are some values that are significant at the $P<0.05$ level or the $P<0.01$ level, many are not significant. These observations differ from the task-dependence in the H-reflex (Capaday and Stein 1986; 1987; Edamura et al. 1991) in which the reflex during standing could be up to 3.5 times larger than during walking and the reflex gain was substantially decreased from walking to running at the same speed (6 km/h). However, some of these results have not been reproduced in a recent study (Ferris et al. 2001). Similarly, dramatic differences are seen in other reflexes. For example, Duysens et al. (1993) compared cutaneous reflex during standing and running, and found a directional difference between the tasks; the response in the TA was predominantly suppressive during standing and facilitatory during running. Similarly, Pearson et al. (Pearson and Collins 1993; Pearson 1995; Pearson et al. 1998) found that the inhibition, arising largely from Golgi tendon organs during standing could be reversed to an excitation during some phases of walking. In the present study, inhibition was never reversed to facilitation across different tasks. We conclude that there is some evidence for a task dependence of reciprocal inhibition, but it is weaker than for other spinal reflexes.

Table 2-1 Task-dependence of reciprocal inhibition

Nerve stimulation	task	difference	data compared
CP (1.5 x MT)	standing vs. walking (4 km/h)	$P < 0.01$	all values
CP (1.5 x MT)	standing vs. walking (4 km/h)	$P < 0.05$	matched EMG levels
CP (1.1 x MT)	standing vs. walking (4 km/h)	NS	all values
CP (1.1 x MT)	standing vs. walking (4 km/h)	NS	matched EMG levels
Tibial (1.5 x MT)	standing vs. walking (4 km/h)	NS	all values
Tibial (1.5 x MT)	standing vs. walking (4 km/h)	NS	matched EMG levels
Tibial (1.1 x MT)	standing vs. walking (4 km/h)	NS	all values
Tibial (1.1 x MT)	standing vs. walking (4 km/h)	NS	matched EMG levels
CP (1.5 x MT)	standing vs. walking (3 km/h)	NS	all values
Tibial (1.5 x MT)	standing vs. walking (3 km/h)	$P < 0.01$	all values
CP (1.5 x MT)	walking (6 km/h) vs. running (6 km/h)	$P < 0.05$	all values
Tibial (1.5 x MT)	walking (6 km/h) vs. running (6 km/h)	NS	all values

The rationale for a task-dependence is clear for these other reflexes. For example, the calf muscles are stretched under the weight of the body during stance and the stretch reflex is useful in pushing the body off the ground at the end of stance. However, the muscles are also stretched during the swing phase, so, without reflex modulation, a reflex would extend the ankle and perhaps cause the foot to hit the ground (Capaday and Stein 1986; Yang and Whelan 1993; Schneider et al. 2000). Similarly, a cutaneous input during swing phase leads to a flexion reflex that can prevent a stumble, but when the limb is striking the ground at the end of stance, a flexion could cause a fall. An extension is needed to support the body (Yang and Stein 1990; Zehr et al. 1997; Zehr et al. 1998; Zehr and Stein 1999). Finally, as long as a limb is loaded during walking, extensors must remain active to support the body weight and positive feedback from Golgi tendon organs would be very useful for this purpose (Pearson 1995; Whelan et al. 1995; Hiebert et al. 1996; Dietz and Duysens 2000; Duysens et al. 2000; Pang and Yang 2000).

2.4.5 Effects of speed and functional role

We cannot envisage a similar functional role for task dependence of a spinal reciprocal inhibition. Although the task dependence was weak, when we examined the full range of speeds in Fig. 2-5, there is a large and consistent decrease in the inhibition with speed. This makes sense since, as the speed is increased, there will be less time in a given phase for the spinal inhibition to act. In addition, for rapid movements many muscles need to be activated maximally. As a muscle is rapidly shortening, muscle spindles in the antagonist muscles will be powerfully stretched, but a reciprocal inhibition from this action would limit the desired activation of the agonist. A similar suppression

of reflex effects has been found in other quite different studies. For example, Brandt (1999; 2000) reported that when subjects were asked to walk or run after a transient vestibular tone imbalance, deviation of gait was more with walking than with running. It was also found that deviation decreased and stability increased from standing, to walking, to running in a dog with acute labyrinthine failure. Thus, the author(s) suggested that a spinal program for locomotion suppresses destabilizing vestibular inputs. Recently, Prochazka et al. (2002) introduced a locomotor model by which contribution of the centrally generated activity and reflex components to locomotor activity can be evaluated. According to their model, stretch reflexes are crucial when the central influence is low, whereas the reflexes are less important when the centrally originated activity level is high. Sensory signals are also modulated during movements (Brooke et al. 1997; Menard et al. 2003). For instance, cutaneous reflexes are influenced by afferents from load receptors during walking (Bastiaanse et al. 2000). Moreover, connection between sensory afferents and interneurons are likely altered during motor tasks under the influence of descending input (Hultborn 2001). Thus, afferents involved in reciprocal inhibitory pathways might be gated to different degrees during locomotion at different speeds.

In conclusion, a weak task-dependence was observed in spinal reciprocal inhibition, and a much stronger dependence was found with speed. This may be functionally important so that muscles can be maximally activated during rapid movement.

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3. SPINAL EXCITATION AND INHIBITION DECREASE AS HUMANS AGE

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3.1 Introduction

The human H-reflex, an electrical analog of the tendon jerk, has been studied extensively and is known to be modulated both within a task and between tasks (Capaday and Stein 1986; Capaday and Stein 1987; Edamura et al. 1991; Yang and Whelan 1993; Brooke et al. 1995; Garrett et al. 1999; Schneider et al. 2000; Trimble et al. 2001). Whether there are systematic effects of aging has been more controversial. For example, the ratio between the maximum H-reflex and M-wave (H_{max}/M_{max}) was significantly smaller in old than in young subjects in some studies (Sabbahi and Sedgwick 1982; Brooke et al. 1989; Koceja et al. 1995; Scaglioni et al. 2002), but not in others (Angulo-Kinzler et al. 1998; Earles et al. 2001). Chalmers and Knutzen (2000) compared the H-reflex modulation during walking between young and elderly subjects and found similar overall modulation with different reflex size in the midstance, even though the magnitude of the H-reflex decreased with age. The posture-dependence of the H-reflex has also been compared between young and old subjects (Koceja et al. 1995; Angulo-Kinzler et al. 1998; Chalmers and Knutzen 2002). Previous studies have only examined groups of young and old subjects. Are any changes that occur gradual or do they only occur above 60 years of age, for example?

The effect of aging on spinal inhibition has been much less studied. Morita et al. (1995) studied heteronymous Ia facilitation from the femoral nerve to the soleus muscle in healthy subjects aged from 24 to 68, and showed a linear decrease of the facilitation with age. They suggested that this decrease might reflect an increase in the presynaptic inhibition on Ia terminals, as well as a decrease in the number and conduction velocity of Ia fibers. Changes in presynaptic inhibition with age were further studied by Earles et al. (2001), using the soleus H-reflex inhibition by CP nerve stimulation. Presynaptic inhibition of the soleus H-reflex was significantly smaller in the elderly than in the young group at rest, and not significant in the elderly at a level of 20% maximum voluntary contraction. Also, Earles et al. (2001) showed lower presynaptic inhibition in the elderly, in contrast to the suggestion by Morita et al. (1995). The above studies have concentrated on presynaptic inhibition, so the effects on reciprocal, postsynaptic inhibition are unknown. Finally, the task-dependence of reciprocal inhibition has been controversial at any age (Capaday et al. 1990; Lavoie et al. 1997; Petersen et al. 1999). This issue has been dealt with elsewhere (Kido et al. 2004).

The main purpose of this study was to investigate the dependence on age of spinal excitatory and inhibitory reflexes in healthy adults. We collected data from subjects aged between 22 and 82, including some at intermediate ages, that have not been well studied. As a result we could test whether spinal reflexes change continuously with age. Also, reflexes were measured during standing and walking to examine the effect of age on the task-dependence.

3.2 Materials and methods

3.2.1 General procedure

Twenty-three neurologically normal subjects aged from 22 to 82 participated in this study. Even the oldest subjects were quite active and walked without the use of walking aids such as a cane. All subjects gave informed consent for the purposes and procedures of the experiments, as approved by the Human Ethics Committee of the University of Alberta. Electromyography (EMG) was recorded from the tibialis anterior (TA) and soleus muscles with surface self-adhesive Ag-AgCl electrodes (Kendall LTP, Chicopee, MA). At the beginning of each experiment, the maximum tonic voluntary contraction (MVC) of each muscle was determined as the maximum rectified EMG level. During standing, the subject was asked to control a target muscle EMG level voluntarily for either plantar flexion or dorsiflexion to match a preset level on an oscilloscope (see the section *EMG recordings and electrical stimulation*). Then, reciprocal inhibition of the ankle extensors and flexors was elicited by stimulating the common peroneal (CP) or the tibial nerve respectively, while the subject was standing upright or walking on a treadmill.

Reciprocal inhibition of the soleus EMG activity was examined by stimulating the CP nerve in 22 subjects. Under fixed tonic contraction (about 15% MVC) of the soleus, the stimulus intensity was varied to determine the motor threshold (MT) of CP nerve stimulation. Collected data were immediately analyzed by a custom-written program in MATLAB (Mathworks Inc., Natick, MA). Then, the effect of soleus contraction level on the amount of inhibition was tested at a variety of soleus tonic contraction levels using 1.5 × MT of the CP nerve stimulation. Although the stimulus intensity of 1.5 × MT is stronger than that used by Petersen et al. (1999), recently we found that 1.5 × MT of

stimulation can produce inhibition more reliably without affecting the task-dependence (Kido et al. 2004). Similarly, inhibition of soleus EMG activity elicited by the CP nerve stimulation was studied during walking. The subject was asked to walk on a treadmill at his/her comfortable speed for 4 min (2.5-4 km/h), while the CP nerve stimulation (1.5 × MT) was applied at all parts of the step cycle with random intervals (1.6 to 2.7 s). The same protocols were used for the tibial nerve stimulation and the inhibition of TA voluntary activity in 21 subjects.

In 23 subjects, the soleus H-reflex was elicited by stimulating the tibial nerve. First, the H-reflexes (and M-waves) were elicited at various stimulus intensities during standing with the soleus tonic EMG activity level of 15% MVC, in order to measure the threshold stimulus intensity and maximum response amplitude. Intensities were varied from the soleus H-reflex threshold to M_{max} (M-H curve, Zehr & Stein, 1999). Four EMG responses were collected at each intensity. Subsequently, a test stimulus that elicited 50% of H_{max}, which was in the range of 5-40% M_{max} (20.0±11.8%, mean ± SD) in different subjects, was applied during walking on a treadmill for 4 min and standing with different soleus tonic contraction levels. Fifty percent of H_{max} was chosen from the rising phase of H-reflexes in the stimulus-response relationship, since this size of H-reflex is sensitive to both inhibitory and facilitatory modulation (Zehr and Stein 1999). Also, in order to examine whether the M_{max} amplitude changes during walking, the soleus M-H curves were measured at different times of step cycle in 2 subjects. The step cycle was divided into 12 equal bins and responses produced by stimuli within the same bin were averaged together.

3.2.2 EMG recordings and electrical stimulation

The soleus and TA EMG signals were obtained using surface self-adhesive Ag-AgCl electrodes (Kendall LTP, Chicopee, MA). EMG recording electrodes were placed 2-3 cm below the gastrocnemius in line with the Achilles tendon for the soleus, and over the motor point for the TA, with ~2 cm interelectrode spacing. The signals were amplified, high-pass filtered at 10 Hz, low-pass filtered at 1 kHz, and recorded with Axoscope (Axon Instrument, Union City, CA) at a sample rate of 5 kHz. EMG signals were also rectified, low-pass filtered at 3 Hz, and sent to an oscilloscope, so that subjects could monitor their EMG activity levels during experiments. During standing EMG and nerve stimulus signals were recorded for 300 ms, including a prestimulus period of 40 ms, in response to each test stimulus pulse. During walking heel contact was detected by a force-sensitive resistor (Interlink, Camarillo, CA) inserted between a subject's shoe and foot, and used to define the beginning of the step cycle.

The electrical stimulation was applied to the CP nerve and/or the tibial nerve by surface Ag-AgCl electrodes (Kendall LTP, Chicopee, MA). The stimulus electrode for the CP nerve was placed at a low threshold point near the neck of the fibula and the anode electrode was located below the patella. The electrodes for the tibial nerve stimulation were placed in the popliteal fossa and over the patella. Stimulus pulses were 1 ms rectangular pulses and delivered from Grass SD9 isolated stimulators (Grass Inst., Quincy, MA). The test stimulation was triggered by a random pulse generator, so that the stimuli were generated with pseudo-random intervals (1.6 to 2.7 s).

3.2.3 Data analysis

To evaluate the effect of antagonist muscle nerve stimulation on the target muscle activity, the mean level of inhibition in the rectified target muscle EMG was measured for a 7-10 ms period including the peak inhibition (Fig. 3-1). When the period of inhibition appeared to be longer than 20 ms, the first 10 ms was used for analyses. Since it was measured over a period of time, it may contain disynaptic and other short latency reflex components. We will refer to this as reciprocal inhibition without specifying pathways that may be involved. To measure the background EMG activity level for standing, a 40 ms prestimulus period was used. For walking, the average EMG activity was computed for unstimulated steps (≅50 steps), and used as the control EMG (Capaday et al. 1990; Yang and Stein 1990; Petersen et al. 1999). The amount of reciprocal inhibition was expressed as the difference in the mean rectified EMG levels in the chosen 7-10 ms period from the background studied for 40 ms prior to stimulation or in the control EMG of unstimulated steps. Some of the responses produced by too strong or too weak stimuli (indicated by amplitudes of M-waves) were eliminated from the further analysis. Later, for comparison across different ages, the amount of inhibition was normalized by the background EMG. The peak-to-peak amplitudes of the H-reflex and M-wave were collected and related to the background EMG level during standing, as described above, and during walking, calculated at the corresponding time in unstimulated steps.

A main purpose of this study was to investigate the effects of aging on excitatory and inhibitory spinal reflexes. Thus, H_{max} and M_{max} were measured during standing with an EMG in the soleus muscle equal to 15% MVC. Modulation of the H-reflex during walking was evaluated using the modulation index (i.e., 100 × (maximum H-reflex –

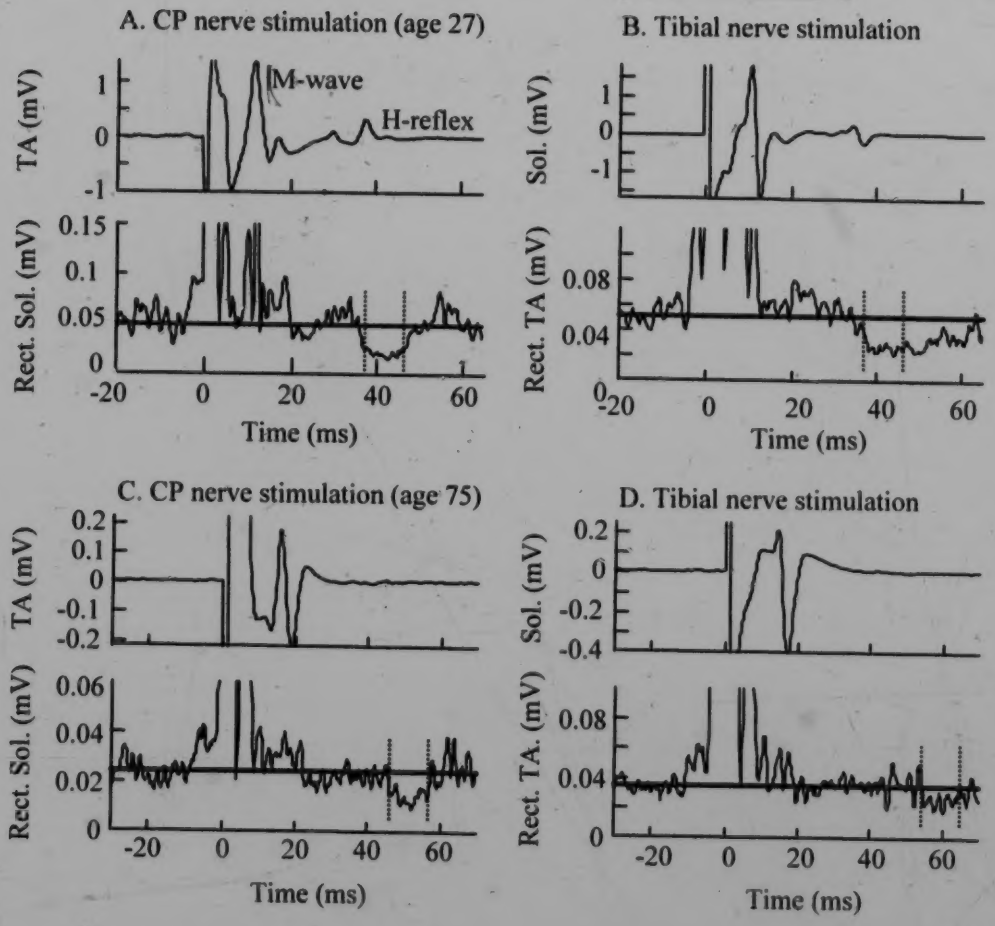

Fig. 3-1 Stimulating an antagonist muscle nerve decreases ongoing voluntary EMG activity. A and C: Reciprocal inhibition of the soleus EMG activity was induced by 1.5 × MT of the common peroneal (CP) nerve stimulation, following the H-reflex in the TA muscle. Note that the positive signal of 0-20 ms in the rectified soleus EMG is pickup from activation of the CP nerve innervated muscles (e.g., extensor digitorum longus and peroneus longus). B and D: Reciprocal inhibition of the TA EMG activity was elicited by 1.5 × MT of the tibial nerve stimulation. The mean level of inhibition (difference between the mean depression and the mean rectified background EMG level) was measured for a 7-10 ms window (see bars) including the peak depression in the rectified EMG. Background EMG levels were calculated from 40 ms of prestimulus period for standing data and from the corresponding 7-10 ms window of unstimulated steps for walking data. Thick horizontal lines indicate background EMG levels. The time 0 refers to time of the nerve stimulation. Twenty sweeps were averaged for each part of the figure. (A and B from subject A age 27, C and D from subject W age 75)

minimum H-reflex) / maximum H-reflex; (Zehr and Chua 2000; Zehr and Kido 2001). Also, the amount of reciprocal inhibition was examined in relation to age. The Pearson's correlation coefficient was calculated between each parameter and age to assess the effect of aging.

3.3 Results

3.3.1 Soleus H-reflex

Examples of H_{max} elicited during standing with a soleus EMG activity of 15% MVC are shown in Fig. 3-2A and B. The subject shown in 3-2A (age 36), had a large H-reflex with a latency of less than 30 ms, following a small M-wave. On the contrary, the subject of 2B (age 82) had a small H-reflex with a latency of approximately 40 ms. Note the different scales used in Fig. 3-2A and B. Small H-reflexes were found not only in this particular subject, but also in other older subjects. Fig. 3-2C shows amplitudes of the soleus H_{max} and M_{max} measured during standing as a function of age. For regression analysis, we calculated a linear correlation since fitted polynomial curves did not give any better correlations. Both M_{max} and H_{max} become smaller with age (r=-0.62 and -0.76, respectively). Also, the H_{max}/M_{max} ratio, plotted against age, showed a significant decrease with age (r=0.79, Fig. 3-2D). Finally, the onset of H-reflex measured during standing with 15% MVC of tonic voluntary activation of the soleus is shown in Fig. 3-3A. The latency becomes significantly longer with age (r=0.78, P<0.01).

Interestingly, despite a progressive decrease of the H-reflex with age, the H-reflex modulation during walking was not much affected by aging. Fig. 3-3B shows phase-dependent modulation of the H-reflex during walking in the same subjects shown in Fig.

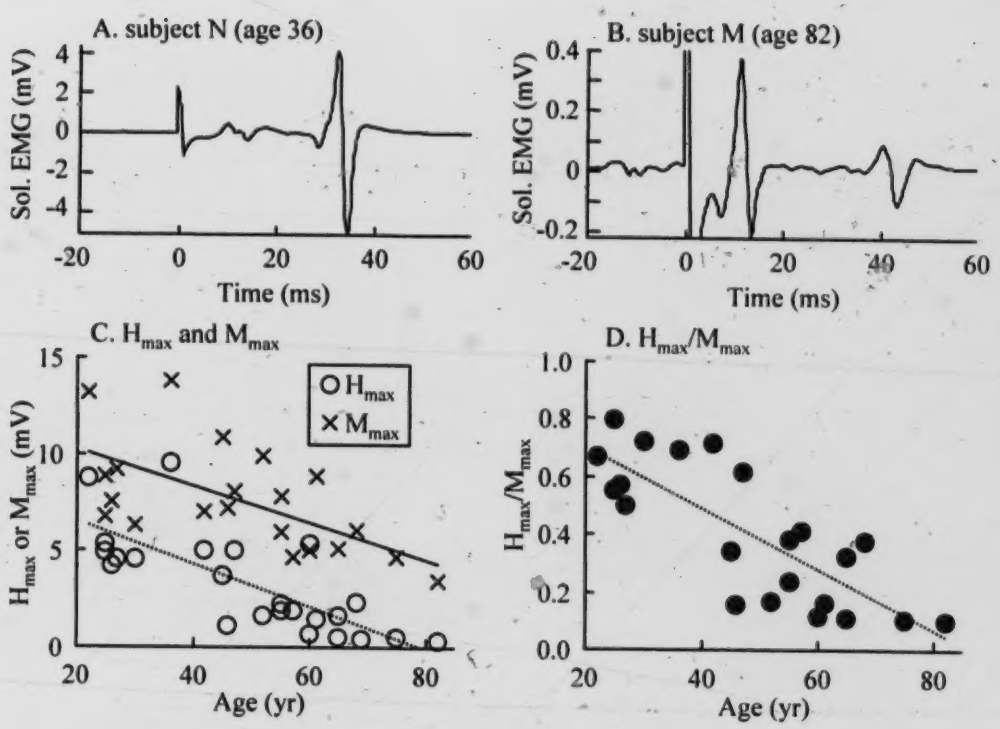

Fig. 3-2 M_{max}, H_{max} and H_{max}/M_{max} decrease with age. A and B: The maximum soleus H-reflexes (H_{max}) elicited during standing with soleus EMG level of 15 % MVC in single subjects are shown. A is from a 36 years old subject and B is from a 82 years old subject. Note that different scales were used for different subjects. Each sweep is the average of 4 responses. C: Amplitudes of soleus H_{max} and maximum M-wave (M_{max}) measured during standing become smaller with age. D: The H_{max}/M_{max} ratio is plotted against age, and shows a significant decrease with age.

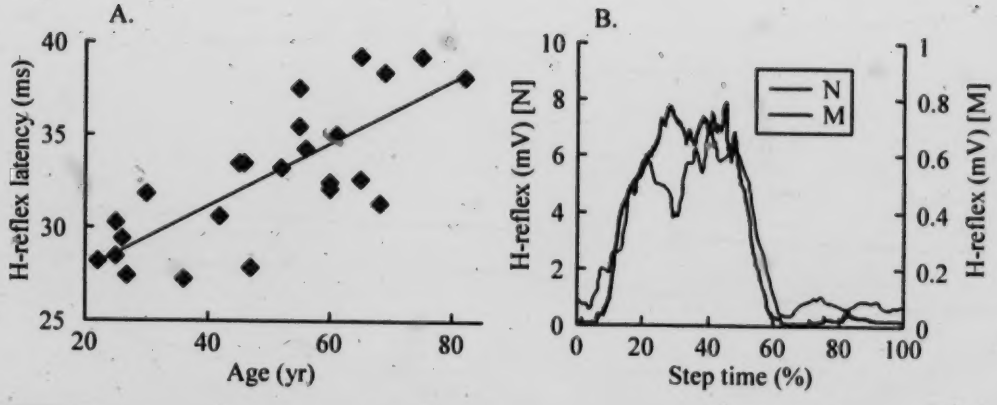

Fig. 3-3 A. The H-reflex latency measured during standing increases with age. B. The soleus H-reflex is modulated with the phase of walking in both young and old subjects. A thin solid line shows the H-reflex in subject M (age 82) and a thick fuzzy line shows the reflex in subject N (age 36). Note that different scales are applied for different subjects. Although the older subject had smaller H-reflexes, overall, her reflex modulation pattern was similar to that of the younger subject.

3-2A and B. Although the 82 year old subject M had much smaller H-reflexes than the 36 year old subject N (compare the right and left y-axes), similar modulation patterns were observed in both subjects. Indeed, all subjects had a modulation index (MI) >92.5 (98.2±1.6), indicating relatively preserved phase-dependency of the reflex throughout the aging process. The lowest values were for subjects over 70 years of age who had very small H-reflexes, so the modulation was more difficult to measure.

The soleus H-reflexes measured during standing and the stance phase of walking are shown in Fig. 3-4. As found by Capaday and Stein (1986), the amplitude of the H-reflex was larger during standing than during walking (Fig. 3-4A). When the mean amplitudes of the H-reflex, calculated for the background EMG of 20-60% MVC, were compared between standing and walking, the difference was significant (group mean ± SD: 3.64±2.21 mV for standing, 1.98±1.46 mV for walking, $P<0.0001$, Student's paired t test). However, as can be seen in an example of a single subject in Fig. 3-4B (age 82), this task-dependent difference in the H-reflex amplitude was less pronounced in older subjects. The difference in the H-reflex between the tasks was significant for the subject of Fig. 3-4A ($P<0.0001$, Student's t test), but not for the subject of Fig. 3-4B ($p = 0.91$). When paired t tests were performed between the tasks for 3 different age groups (i.e., <40, 40-59, ≥60), the difference was significant for all three groups (<40; n=7, $P<0.001$, 40-59; n=8, $P<0.05$, ≥60; n=7, $P<0.05$), although the significance was less in the elderly group. Fig. 3-4C shows the mean amplitudes of the soleus H-reflex as a function of age. Slopes of regression lines fitted were not significantly different between standing and walking ($P>0.05$).

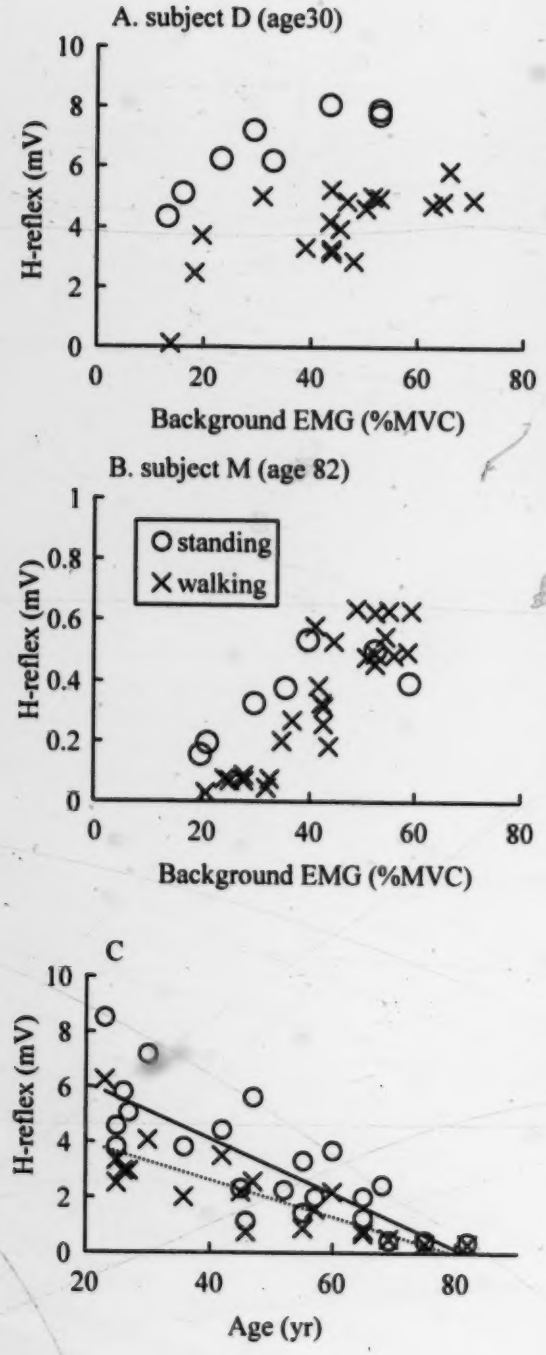

Fig. 3-4 A and B: The soleus H-reflexes measured during standing and the stance phase of walking are shown as a function of the soleus background EMG levels in single subjects. The H-reflexes are the means of the closest four data points in the step cycle for walking and in the background for standing. Note the clear difference in the H-reflex amplitude between the two tasks in the young subject (A), but not in the older subject (B). C: The mean amplitudes of the soleus H-reflex during standing and walking were calculated for the background EMG level of 20-60% MVC in each subject and pooled to show the effect of age. Slopes of regression lines fitted to H-reflexes during standing (solid line, r=0.80) and walking (dotted line, r=0.79) were not significantly different.

In order to examine the extent of change in the M_{max} during walking, the soleus M-waves and H-reflexes were collected at different time of the step cycle with different tibial nerve stimulus intensities. Soleus M-wave amplitudes measured in single subjects are presented in Fig. 3-5A-D. Fig. 3-5A and B show the M-response curves in standing, while Fig. 3-5C and D show the curves in 12 equally separated bins of the step cycle. Approximately 10 responses were averaged for each bin at a stimulus intensity. Over the step cycle changes in the M_{max} were very small: ± 6.5% in subject A (Fig. 3-5C) and ±5.7% in subject R (Fig. 3-5D). The M_{max} and the H_{max} are compared between standing and walking in Fig. 3-5E and F. While the M_{max} during walking was quite stable around the value during standing, the H_{max} was greatly modulated across the step cycle.

3.3.2 Reciprocal inhibition of ongoing EMG activity

Stimulation of an antagonist muscle nerve produced inhibition of tonic voluntary EMG activity (Fig. 3-1). Examples of the soleus inhibition induced by 1.5 × MT of the CP nerve stimulation are shown in A and C, and the TA inhibition by 1.5 × MT of the tibial nerve stimulation in B and D. Following appearance of the H-reflex in the antagonist muscle, ongoing voluntary EMG activity decreased in the target muscle (see Fig. 3-1A and B from subject A, age 27). Note that although the TA H-reflex (>0.1 mV) could only be evoked in a few subjects (n=3), the amplitude was often small, and inhibition was observed even in subjects without clear H-reflexes. Thus, the presence or absence of the H-reflex did not affect the amount of inhibition. Fig. 3-1C and D are from subject W, aged 75, showing no H-reflexes but some inhibition.

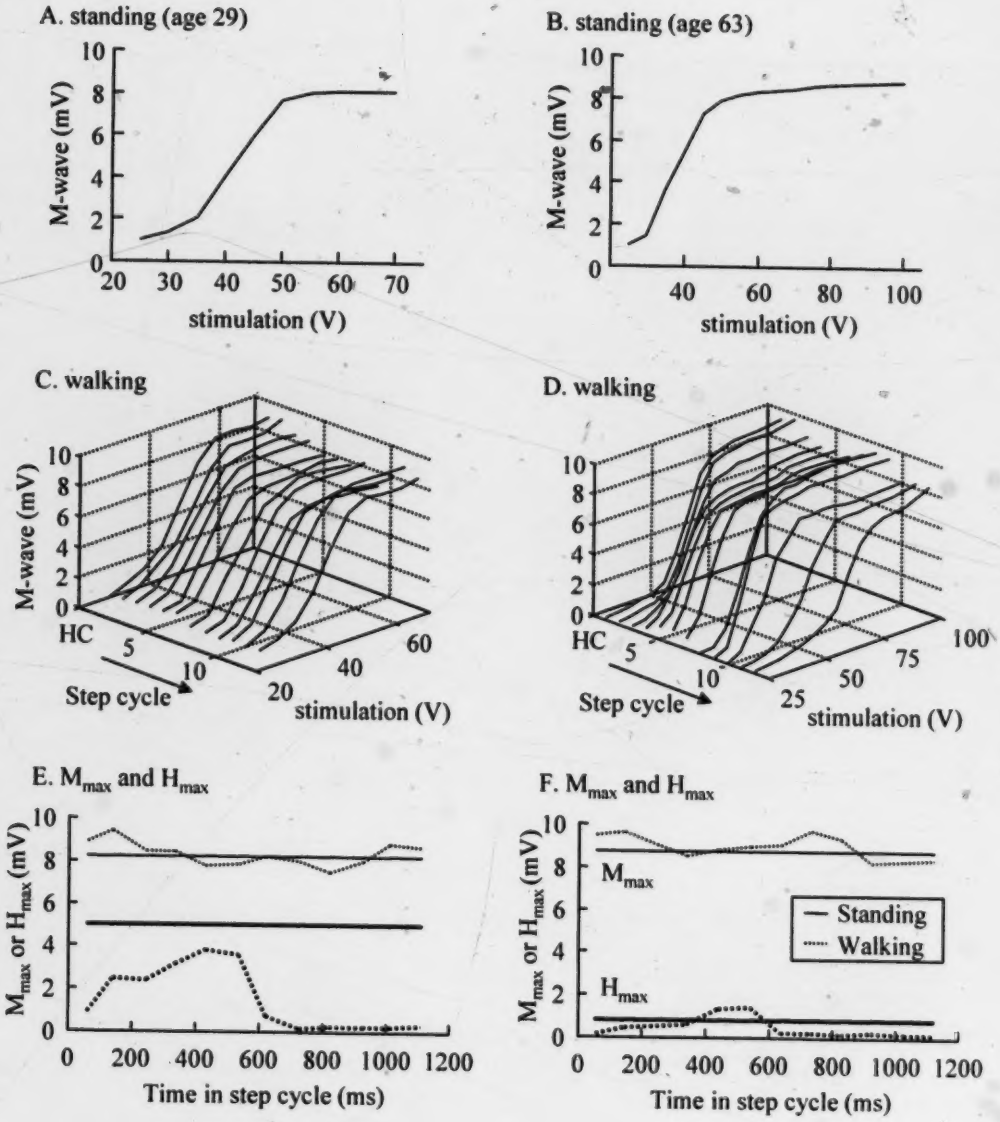

Fig. 3-5 The soleus M-H curves were measured during standing and walking in two subjects. A and B: M-wave amplitudes during standing with 15% MVC of soleus activation. C and D: M-wave amplitudes during walking. The step cycle was divided into 12 equal bins and responses elicited by stimuli within the same bin were averaged together. Heel contact (HC) was marked as the beginning of the step cycle. E and F: The M_{max} and H_{max} during standing and walking. A, C, and E are from subject A (age 29) and B, D, and F are from subject R (age 63).

In Fig. 3-6 reciprocal inhibition of ongoing EMG activity is plotted against different background EMG levels. A dashed line drawn in each panel corresponds to 100% inhibition. The higher the background EMG level, the larger the amount of inhibition for both the soleus and TA. In the subject of Fig. 3-6A and B (age 30), slopes of regression lines were 0.63 for standing and 0.42 for walking in the soleus and 0.79 for standing and 0.70 for walking in the TA. In the subject shown in Fig. 3-6C and D (age 75), slopes were 0.38 for standing and 0.31 for walking in the soleus and 0.32 for standing and 0.24 for walking in the TA. The difference in slopes between these two subjects suggests an effect of age on the amount of reciprocal inhibition.

For inhibition of the soleus EMG, the correlation coefficient was significant in 21 of 22 subjects examined for standing (mean ± SD: 0.83±0.16) and in 19 of 22 for walking (0.64±0.21). Slopes of regression lines were significantly greater than 0 for both standing (0.469±0.126, $P<0.0001$; Student's one sample t-test) and walking (0.280±0.123, $P<0.0001$), indicating significant effects of background EMG level on the amount of inhibition. This also means that the dependence of the inhibition on background activity was present at all ages examined. As very little inhibition could be observed at a background EMG level below 20% MVC and there were not too many data points over 60% MVC level, the data from background EMG levels between 20-60% MVC were used for the rest of analyses. The soleus EMG level was too low during the swing phase of walking (i.e., below 20% MVC level) to be included in this range. For comparison across different ages, the amount of inhibition was normalized by the background EMG amplitude and the mean inhibition was calculated for 20-60% MVC of background EMG in each subject. In the subject shown in Fig. 3-6A, 52.2% of the background activity was

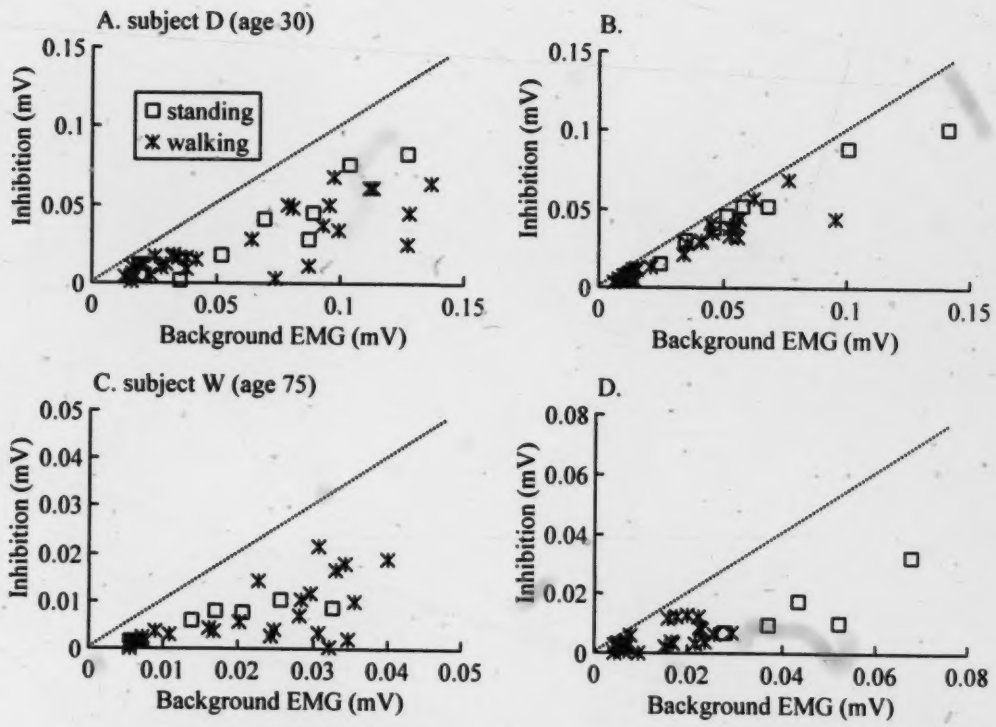

Fig. 3-6 Inhibition of ongoing EMG activity increases as the background EMG activity increases. The amount of inhibition is the difference between the mean rectified EMG (mrEMG) of a selected 10 ms window and the background (BG) EMG. It is presented as a function of BG EMG levels for standing and walking. Dashed lines are unity lines indicating complete suppression of ongoing EMG activity. A and C: Reciprocal inhibition of the soleus EMG elicited by 1.5 × MT of the CP nerve stimulation. B and D: Reciprocal inhibition of the TA EMG elicited by 1.5 × MT of the tibial nerve stimulation. A and B are from a young subject, whose soleus and TA MVC EMG amplitudes were 0.23 mV (for both). C and D are from an older subject, whose soleus and TA MVC EMG amplitudes were 0.06 mV and 0.1 mV.

inhibited (i.e., the normalized inhibition was 0.522) in standing.

For the TA inhibition, the correlation coefficient was significant in 19 of 21 subjects examined for standing (mean ± SD: 0.82±0.17) and in 14 of 21 for walking (0.60±0.30). Of 7 subjects who did not show a significant linear correlation between the inhibition and background activity level during walking, 6 were over 60. Thus, aging may have some effects on the background activity-inhibition relationship. However, we cannot exclude effects from other factors such as daily physical activity. Slopes of regression lines were significantly greater than 0 for both standing (0.491±0.188, $P<0.0001$; Student's one sample t test) and walking (0.386±0.226, $P<0.0001$), indicating significant effects of background EMG level on the amount of inhibition. As little inhibition was present at background EMG levels below 10% MVC and there were not too many data points over 50% MVC level during walking, the data for the 10-50% MVC background EMG level was normalized by the background EMG amplitude and used for the rest of the analyses. The TA EMG level was too low in most of the stance phase of walking to be included in this range (i.e., 10-50% MVC).

Reciprocal inhibition of the soleus EMG activity induced by CP nerve stimulation at 1.5 × MT is expressed as a function of age in Fig. 3-7A. The amount of inhibition, calculated for the background EMG level of 20-60% MVC, decreases with increasing age in standing (r=-0.57, slope =-0.004, $P<0.01$) and walking (r=-0.61, slope =-0.005, $P<0.01$). Regression lines were not significantly different between standing and walking; differences in slopes and intercepts were not significant ($P>0.05$). Inhibition of the TA ongoing EMG by tibial nerve stimulation at 1.5 × MT in all subjects is pooled in Fig. 3-7B. The amount of inhibition, normalized and averaged for the background EMG

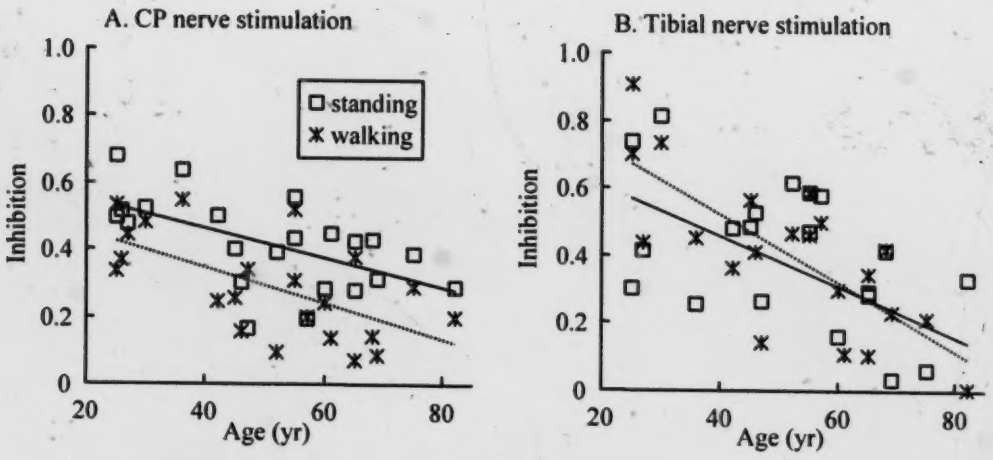

Fig. 3-7 Reciprocal inhibition of the ongoing EMG activity by 1.5 × MT of the antagonist muscle nerve stimulation in all subjects is shown as a function of age. Solid lines indicate the trends for standing and dotted lines for walking. A: Inhibition of the soleus EMG activity decreases with age in both standing and walking. The amount of inhibition, calculated as the difference between the mean rectified EMG of a selected 10 ms window and background (BG) EMG, is normalized by the BG and averaged for the BG EMG level of 20-60 % MVC. B: Reciprocal inhibition of the TA EMG activity, normalized and averaged for the BG EMG level of 10-50 % MVC, decreases with age in both standing and walking.

level of 10-50% MVC, decreases with age in both standing (r=-0.47, slope =-0.008, P<0.05) and walking (r=-0.75, slope =-0.01, P<0.01). Regression lines were not significantly different between standing and walking (P>0.05). In general, the relative amount of inhibition decreased with age in both the CP and tibial nerve stimulation, as indicated by the significant linear regression.

3.4 Discussion

The purpose of this study was to investigate spinal excitatory and inhibitory reflexes in healthy adults at different ages. The soleus H-reflex and reciprocal inhibition of ankle extensors and flexors measured as inhibition of ongoing EMG activity were examined during standing and walking, and expressed as a function of age. The soleus H_{max} decreased linearly with age, accompanied by a smaller decrease of M_{max}, producing a pronounced decrease in the ratio of H_{max}/M_{max}. Task-dependent modulation of the H-reflex was less pronounced in older subjects, while modulation within a task (i.e., phase-dependent modulation) during walking was present across different ages. The amount of reciprocal inhibition in the soleus and TA also decreased with age, and this age-related decrease of inhibition was significant in both standing and walking.

3.4.1 Effects of aging on the soleus H-reflex

Crone and her colleagues proposed (Crone et al. 1985; Crone et al. 1987; Crone and Nielsen 1989; Morita et al. 2001) that a stimulus intensity producing 15-25% M_{max} of H-reflex amplitude is the best to observe any modulation. In this study the stimulus intensity was chosen to produce the H-reflex amplitude of 50% H_{max} (Zehr and Stein

1999), rather than fixed ratio to M_{max}. The reason for this was that our main interest was to examine the effect of age and the amplitude of H-reflex has been reported to be small in the elderly (Sabbahi and Sedgwick 1982; Brooke et al. 1989; Koceja et al. 1995; Scaglioni et al. 2002). Thus, we thought that in older subjects the H_{max}/M_{max} ratio might be less than 0.15 (i.e., H_{max} < 15% M_{max}) and it would be better to determine the stimulus intensity according to the size of H-reflex elicited. Indeed, this was the case. In the present study, 5 of the subjects over 60 had the ratio of H_{max}/M_{max} <0.15 (see Fig. 3-2), and for those a stimulus intensity eliciting an H-reflex of 5-8% M_{max} was used.

On eliciting excitatory and inhibitory reflexes during movement, it is important to maintain the number of afferent axons activated across different conditions, and this has been achieved by monitoring the M-wave amplitude (Brooke et al. 1997; Capaday 1997; Zehr 2002; Misiaszek 2003). However, if the M_{max} changes during movement, maintaining the constant M-wave amplitude is no longer appropriate since the proportion of neurons recruited would also change. During walking and running, however, Ferris et al. (2001) found that the M_{max} changed only up to 20-30% over the step cycle. Furthermore, in the present study, the M_{max} showed a small change (±5-7%) over the step cycle (Fig. 3-5). Also, while the H_{max} was greatly modulated across the step cycle, as previously observed (Capaday and Stein 1986; Capaday and Stein 1987; Edamura et al. 1991; Yang and Whelan 1993; Brooke et al. 1995; Garrett et al. 1999; Schneider et al. 2000; Trimble et al. 2001), the M_{max} during walking was similar to the value during standing throughout the step cycle. Thus, we believe that in the present study the stimulus intensity could be kept reasonably constant by maintaining the size of M-wave throughout an experiment.

The latency of H-reflex measured during standing increased with age (Fig. 3-3A). This agrees with observations by Sabbahi and Sedgwick (1982), Scaglioni et al. (2002), and Brooke et al. (1989). Some variation in the latency might be explained by the variation in leg length among subjects (Falco et al. 1994a), but there was no systematic trend with age in the height of our subjects. Another possibility is a decrease in conduction velocity, but a 10 ms change is too large to be explained in this way (Rivner et al. 2001). Since the H-reflex is getting smaller, the most likely cause is an increased time for the EPSPs produced by Ia afferents to reach threshold (Chase et al. 1985; Boxer et al. 1988; Hui-Chan and Levin 1993).

Amplitudes of soleus H_{max} and M_{max} measured during standing with 15% MVC of the soleus EMG activity decreased linearly with age (Fig. 3-5). Also, the H_{max}/M_{max} ratio decreased with age, since the decrease in the H_{max} was larger than that in M_{max}. These results agree with the observation by Sabbahi and Sedgwick (1982), Brooke et al. (1989), Koceja et al. (1995), and Scaglioni et al. (2002), in which the H_{max}/M_{max} ratio was significantly lower in the elderly than in the young. The fitted straight line in Fig. 3-2D suggests for the first time that the decrease is continuous and gradual over the entire range of ages studied. We also tried fitting a parabola, but the fit was not significantly improved. Thus, we have no evidence that the reflexes change in a curvilinear way (e.g., remaining steady throughout middle age, and then decreasing dramatically in old age). Although some studies have not found a significant decrease with age during standing (Angulo-Kinzler et al. 1998; Chalmers and Knutzen 2000), the same trends were present and significant differences were seen in other postures (Angulo-Kinzler et al. 1998) or in the stance phase of walking (Chalmers and Knutzen 2000). Earles et al. (2001) reported

no difference between standing and walking, but "this was achieved through careful manipulation of stimulus intensity during the onset of volitional contraction." Thus, the evidence is overwhelmingly in favor of some age-related changes in the H-reflex.

Although the H-reflex amplitude decreased with age, the modulation of the H-reflex during walking was essentially the same (i.e., close to 100%) at all ages. Chalmers and Knutzen (2000) also noted the similarity in the overall modulation pattern during walking between young and old subjects, although the H-reflex amplitude was lower in midstance in old subjects. Thus, the pathways that modulate the H-reflex amplitude during walking are relatively preserved while the amplitude of the reflex decreases with age.

In contrast to presence of phase-dependent modulation of the H-reflex, task-dependent modulation of the H-reflex was less pronounced in older subjects. A similar difference in task-dependency between different ages has been found by Chalmers and Knutzen (2002). These authors compared the soleus H-reflex during lying, natural standing, and tandem stance in young and elderly subjects, and found different modulation between the two groups. While young subjects showed differences in the H-reflex amplitude with the order of lying > natural standing > tandem stance, elderly subjects showed no significant difference between lying and natural standing, and significantly smaller reflexes in tandem stance. Similarly, Koceja et al. (1995) studied the H-reflex in standing and prone conditions, and found different task-dependent modulation between young and old subjects. That is, old subjects generally did not show the difference in the H-reflex between two postural conditions. However, small differences in the H-reflex between standing and walking were observed in a few elderly

subjects in the present study, and the difference was significant in a 60 years old subject. Thus, changes in task-dependent modulation probably occur gradually with age, but the primary effect is the overall decrease of the H-reflex with age.

Several physiological changes could contribute to the decrease in the size of the H-reflex with age: 1) a decrease in the number of spinal α-motoneurons (Doherty et al. 1993; Luff 1998), 2) a reduced transmission between Ia afferents and α-motoneurons, 3) an altered presynaptic inhibition (Morita et al. 1995; Earles et al. 2001), 4) a reduced excitability of the α-motoneuron due to decreased spinal and/or supraspinal inputs (Schieppati 1987; Hiersemenzel et al. 2000) and 5) a smaller sensory volley (i.e., reduced Ia afferent action potentials; (Falco et al. 1994b; Rivner et al. 2001).

In the present study, the age-related decrease in the size of the H-reflex was greater than the decrease in the M-wave, and was thus more than expected from a loss of motoneurons. The transmission between the Ia afferents and motoneurons (Ia excitatory postsynaptic potentials, EPSPs) in older animals was longer in the rise-time and half-width, but not different in amplitude compared to ones in young animals (Chase et al. 1985; Boxer et al. 1988). Thus, reduced Ia EPSPs do not seem to be consistent with a large decrease in the H_{max}/M_{max} ratio. Aging effects on spinal presynaptic inhibition has been suggested by Morita et al. (1995) and Earles et al. (2001). Morita et al. (1995) studied the effect of aging on heteronemous Ia facilitation from the quadriceps to soleus, and found a linear decrease of facilitation with age. These authors argued that the decrease of facilitation is attributed to increased presynaptic inhibition. Earles et al. (2001) investigated presynaptic inhibition of the soleus H-reflex by applying conditioning stimulation to the CP nerve at different levels of the soleus voluntary contraction. In their

study the smaller amount of inhibition at rest and a lower presynaptic inhibition gain were found in elderly subjects. Different conclusions drawn from these two studies might be due to different protocols used, which possibly led to examining different types of presynaptic inhibition. Although directions of changes were not consistent between these two studies, both studies indicated altered presynaptic inhibition of the H-reflex with age. Therefore, possibilities 3), 4) and 5) above seem more likely to produce the age-related reduction of the H-reflex.

3.4.2 Effects of aging on inhibition of ongoing EMG activity

This study showed for the first time that the amount of reciprocal inhibition decreases with increasing age in both the soleus and TA, when measured as an inhibition of ongoing EMG activity (Fig. 3-7). Also, the decrease of inhibition was significant in both standing and walking, suggesting there is little compensation for reduced inhibition with age during walking. Again, a parabola did not give a significant improvement in fit, so we have no evidence of a curvilinear relation between inhibition and age. The neural mechanisms are not known, but this age-related decrease of reciprocal inhibition may be related to changes in the transmission efficacy at the Ia afferent terminal on the Ia inhibitory interneuron and/or the terminal of the Ia interneuron on the motoneuron.

In summary, the soleus H_{max} decreased linearly with age, accompanied by a smaller decrease of M_{max}, producing a pronounced decrease in the ratio of H_{max}/M_{max}. The amount of reciprocal inhibition in the soleus and TA also decreased with age. Finally, heteronymous facilitation (Morita et al. 1995) and oligosynaptic reflexes (Brooke et al. 1989) decrease with age, suggesting a general decrease in the excitability of spinal

reflexes with age. While the H-reflex amplitude decreased with age, the modulation of the H-reflex during walking was essentially the same at all ages, suggesting the pathways that modulate the H-reflex amplitude during walking are relatively preserved during the aging process. These results should be considered in future studies, in which the EMG responses are compared between control subjects and patients; the effect of natural aging must be dissociated from abnormalities due to specific disorders. Also, we suggest that studying spinal reciprocal inhibition by conditioning the soleus H-reflex is more problematic than using the depression of ongoing EMG by antagonist nerve stimulation, especially when patients or older subjects are involved. Although conditioning the H-reflex is one of the most commonly used methods to test spinal inhibition, it may not be suitable for the elderly population since the size of the H-reflex itself decreases with age and inhibition of H-reflexes is quite dependent on the test reflex size (Crone et al. 1985; Crone et al. 1990).

3.5 References

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4. ELECTRICAL STIMULATION OF THE HUMAN COMMON PERONEAL NERVE ELICITS LASTING FACILITATION OF CORTICAL MOTOR-EVOKED POTENTIALS

(The author performed all the experiments and largely contributed to data analysis and preparation of the manuscript. A version of this chapter has been published by Michael E. Knash, Aiko Kido, Monica Gorassini, K. Ming Chan, and Richard B. Stein in Experimental Brain Research 153: 366-377, 2003.)

4.1 Introduction

The human sensorimotor cortex is a highly plastic structure. Studies have shown that chronic use (Elbert et al., 1995) or amputation of a body part (Chen et al., 2002; Irlbacher et al., 2002) can induce long-term reorganization of the motor cortex controlling the affected body part. Many recent studies have also reported on the phenomenon of cortical changes being induced over a period of minutes to hours, rather than days to weeks (Stefan et al., 2002; Khaslavskaia et al., 2002; Kaelin-Lang et al., 2002; Ridding and Taylor, 2001; Nielson et al., 1997; Hamdy et al., 1998; Ziemann et al., 2001). One such example is use-dependent plasticity. Training for 30 min is capable of reversing the direction of thumb twitches induced by transcranial magnetic stimulation (TMS). This effect lasted for 15-20 min (Classen et al., 1998). However, short-term plasticity is not limited to voluntary movements as peripheral nerve stimulation can increase corticospinal excitability. For example, two hours of ulnar nerve stimulation at an intensity just above motor threshold, increased the motor evoked potentials (MEP) in muscles innervated by the ulnar nerve for up to 20 min (Kaelin-Lang et al., 2002). Studies comparing TMS and transcranial electrical stimulation (Ridding et al., 2000) or subcortical magnetic

stimulation (Ridding et al., 2001) following repetitive nerve stimulation suggest these effects are due to cortical plasticity.

Previous short-term plasticity studies have largely examined the importance of afferent activation. Khaslavskaia et al. (2002) stimulated the common peroneal (CP) nerve with 5 pulses at 200 Hz every second for 30 min, which resulted in a significant increase in the MEP of the tibialis anterior (TA) muscle. Human motoneurones of the lower limbs rarely fire action potentials at above 30 Hz (Macefield et Al., 1993). Although motoneurones may be capable of following a 200 Hz stimulation pattern, this frequency is actually within the physiological firing rate range of afferent fibres and well above that of motoneurones. Thus, such a stimulation protocol may preferentially activate sensory fibres with higher intrinsic firing rates. We hypothesized that a stimulation frequency of 25 Hz would activate motoneurones effectively and be less effective for sensory fibres than high-frequency stimulation. Can the facilitation of the MEP evoked by high-frequency sensory stimulation be reproduced using a low-frequency, more motor-dominated stimulation protocol? Efferent activity may affect the cortex by inducing muscle contraction, resulting in a natural pattern of afferent input. As plasticity of the human motor cortex is dependent on stimulus parameters such as frequency, intensity, and stimulation time (Fraser et al., 2002) we investigated the effects of 25 Hz electrical stimulation of the CP nerve on the TA MEP, since this frequency is within the physiological firing rate of motoneurones innervating the muscles of the lower leg (Macefield et al., 1993). Peripheral nerve stimulation at 25 Hz is also clinically relevant, as it is the frequency of functional electrical stimulation (FES) used to treat foot drop in stroke patients (Wieler et al., 1999). Recently, Sinkjaer and Khaslavskaia (2002) also

presented a low frequency CP stimulation study in an abstract. They demonstrated an increase in MEP amplitude in relaxed TA following repetitive electrical stimulation of the common peroneal nerve matched with volitional contraction of TA. A number of patients have provided anecdotal evidence suggesting that the ability to dorsiflex the ankle is improved immediately following FES.

The CP is a mixed nerve, so stimulation activates motoneurones, cutaneous afferents, and muscle afferents. Possible mechanisms for potentiating the MEP include: efferent discharge causing a potentiation at the level of the spinal motoneurone, neuromuscular junction, or the muscle fibre itself. Alternatively, afferent input to the cortex could induce disinhibition or increase excitability. We attempted to determine if activity in one or more afferent groups was responsible for facilitating the MEP. For example, vibrating the TA tendon will preferentially activate Ia muscle spindle afferents in this muscle (Kaji et al., 1995; Capaday and Cooke, 1981). The superficial peroneal nerve is a cutaneous branch of the CP nerve innervating the dorsum of the foot. Physiologically, cutaneous excitation on the dorsum of the foot during gait elicits a "stumble correction" (including TA contraction) via the superficial peroneal nerve (Zehr et al., 1998). Therefore, the superficial peroneal nerve was selected as the target nerve for our cutaneous studies.

4.2 Materials and methods

Fourteen healthy subjects (6 female, 8 male) aged 21-62 years (31.3±11.3 years; mean ± SD) without known neurological disorders volunteered for this study and gave informed consent. The project received approval from the University Health Research

Ethics Board. Subjects were seated in a comfortable chair for the duration of the experiment, approximately two hours. The right foot was strapped to a foot plate. Generally the sitting position of the subject was 100° of hip flexion, 40° of knee flexion, and 10° of ankle plantarflexion.

The skin surface over the TA muscle was cleaned with alcohol and allowed to dry before applying disposable, silver/silver chloride recording electrodes (3.3 × 2.2 cm; Kendall, Chicopee MA) over the belly of the muscle. The recording electrodes, as well as an earth electrode, were attached to an isolated pre-amplifier/amplifier (Octopus, Bortec Technologies, Calgary, AB) and amplified 2000-5000 times. The electromyogram (EMG) was filtered (30-1000 Hz, RC filters), digitized (DigiData 1200 Series, Axon Instruments, Union City, CA), and displayed on a personal computer running AxoScope 8 with a 5 kHz sampling rate. The TA EMG was also rectified, low-pass filtered after rectification (3 Hz) and displayed on an oscilloscope. Subjects used this rectified signal to maintain a steady level of voluntary contraction. The foot plate was coupled to a bending-beam load cell (Omega, Stamford CT). The force exerted on the beam was not converted to torque because the distance to the centre of rotation was difficult to determine exactly. Most values were normalized to the initial values, so the % changes would be the same for force or torque.

The best location for stimulating the CP nerve was determined by exploring the skin surface near the neck of the fibula with a stimulating probe. Responses to single electrical pulses (SD9 Stimulator, Grass Instruments, West Warwick RI) were visualized in real time on AxoScope. The stimulation location used was one that consistently produced the largest M-waves (muscle activation resulting from direct orthodromic

axonal conduction) in the TA muscle at the lowest intensity of CP stimulation. Two infant silver/silver chloride disposable electrodes (2.2cm × 2.2cm; Jason, Huntington Beach CA) were placed on the skin to stimulate this location.

The amplitude of the M-wave was measured as a function of the stimulus intensity in Volts (M-wave intensity curve) for each subject (n=10) in order to determine the M-wave threshold (MT), the amplitude of the maximal M-wave (M_{max}), and the stimulus intensity that gave 50% M_{max}. During stimulation of the CP nerve an H-reflex may also be elicited in the TA muscle. Since H-reflexes result from Ia afferent input onto motoneurones, they were analyzed over the course of the study to determine if the excitability of motoneurones was modulated (n=5). Subjects were also asked to perform a maximum voluntary contraction (MVC) of TA and soleus. The scale of the rectified EMG on an oscilloscope was adjusted so that one division corresponded to approximately 15% of the subject's MVC. The rectified EMG was filtered with a low-pass RC filter and displayed to allow subjects to maintain this level of contraction, when requested. Pre-stimulus EMG was analyzed to ensure subjects maintained a constant background contraction level of the muscle being studied across experimental times. Ridding and Rothwell (1995) suggest that although corticospinal plasticity may be evident at rest, it may disappear during voluntary activity, leading the authors to cast doubt on the functional benefits of such phenomena. By performing TMS during volitional activity, we attempted to demonstrate rapid plasticity in a functionally relevant manner.

4.2.1 Repetitive nerve stimulation

Subjects received 20 pulses at 25 Hz (1 ms pulse width) over 800 ms with a 50% duty cycle while sitting passively (no voluntary activity). This pattern continued in 10 min blocks for a total period of 30 min. The stimulation pattern was established by the SD9 Grass Stimulator coupled to a Hewlett-Packard 3300A Function Generator (Palo Alto CA). The initial stimulus intensity was set to produce 50% M_{max}, as determined from the M-wave intensity curve. EMG responses were monitored so the stimulus intensity could be adjusted to maintain an M-wave of approximately 50% M_{max}.

4.2.2 Transcranial magnetic stimulation

TMS was used to evaluate the excitability of the corticospinal pathway. TMS was delivered by a MES-10 Magneto-Electric Stimulator (Cadwell, Kennewick, WA) attached to custom-made, double-D coils with radii of 8 cm and an angle between the coils of 115°. The vertex was identified and marked. A grid was centered over the vertex allowing experimenters to move the coil by 1cm increments. The center of the coil was placed on the scalp 0-2 cm lateral to the vertex with the handle pointing backwards parallel to the midline of the brain. The best location for deliv[redacted]MS in each subject was determined by moving the coil by 1 cm increments on the side contralateral to the recorded TA. The location consistently producing the largest MEPs at the lowest intensity was selected as the site for TMS for the remainder of the experiment. This location ranged in different subjects from 1 cm anterior to 1 cm posterior and 0-2 cm lateral to the vertex. The coil was manually maintained by one of the experimenters so that the position and orientation of the coil were kept constant throughout the experiment. MEPs from TA were displayed in real time on a monitor.

4.2.3 Experimental protocol

Subjects were instructed to maintain a level of contraction corresponding to 15% MVC in order to facilitate the descending cortico-spinal projections when obtaining an MEP input-output curve. Pre-stimulus EMG was analyzed to ensure background contraction levels remained constant across recording times. Subjects received 4 stimuli at a single TMS intensity, with a variable inter-stimulus interval of 3-4 s. Following 4 stimuli the intensity was increased by 10% of the maximum stimulator output and the process was repeated until the MEP did not increase further. The range of intensities used was adjusted for each subject in an attempt to obtain the foot, slope, and plateau of the sigmoidal input-output curve. Force measurements were concurrently performed during the TMS input-output curve.

Once the initial recordings of the M-wave and MEP input-output relations were completed, subjects received repetitive electrical stimulation of the CP nerve. The 25 Hz stimulation continued for 10 min during which time recordings of EMG and force were made every 2 min. After 10 min measurements of M-wave and MEP input-output curves were repeated. Subjects received two more 10 min blocks of stimulation for a total stimulation time of 30 min. At the end of each stimulation block and at three 10 min intervals after stimulation M-wave and MEP input-output curves were recorded to monitor changes in the cortico-spinalmuscular axis.

In addition to TA MEP, responses from soleus were recorded in four subjects to determine if repetitive CP nerve stimulation elicits effects specific to TA or results in a nonspecific global effect. Values of TA MEPs were also obtained at the lowest TMS

intensity capable of consistently evoking responses with subjects at rest (Khaslavskaia et al., 2002). Although resting MEPs showed similar trends as facilitated MEPs, resting MEPs were more variable and a less reliable method for estimating changes in excitability.

4.2.4 Tendon vibration

Muscle afferents were preferentially activated in a group of 5 subjects using 80 Hz tendon vibration (Kaji et al., 1995; Capaday and Cooke, 1981). Eighty Hz was selected as the optimal vibratory frequency as it has been shown to be highly effective in activating muscle afferents (Kosseve et al., 2001). A vibrator was placed at the distal anterior aspect of the lower leg over the TA tendon, while subjects were at rest. An auditory signal was used to time the vibratory presentation, assisting the experimenter to follow a 50% duty cycle (i.e. 800 ms on and off). After initial input-output curves were obtained, vibration was presented in 10 min blocks for a total stimulation time of 30 min with short breaks to record MEP input-output curves. These curves were repeated every 10 min for the next 30 min after stimulation.

4.2.5 Cutaneous stimulation

A group of 5 subjects was also studied to determine the effects of stimulating the superficial peroneal nerve on the MEP. With the right foot strapped to a foot plate, subjects were given a stimulating probe and instructed to explore the area of the anterior aspect of the leg near the talocrural joint, until the sensation changed from one localized to the site of the probe to one radiating across the dorsum of the foot. The place where a

radiating response occurred at the lowest threshold was used subsequently for stimulating the superficial peroneal nerve. A silver/silver chloride electrode was placed at this location with a second electrode on the lateral maleolus. Subjects received repetitive stimulation of the superficial peroneal nerve at 2 times the radiating threshold, with a stimulation pattern analogous to the protocol for the CP nerve. The stimulation frequency was set to 50 Hz to compensate for the smaller number of cutaneous sensory fibres in the superficial peroneal, compared to the CP nerve. This adjustment was only approximate, but was intended to produce a comparable amount of cutaneous input as inferred by subjective sensation. Fifty Hz stimulation was selected after several preliminary experiments employing 25 Hz superficial peroneal stimulation showed no clear effects on MEP. The stimulation again continued with a 50% duty cycle in 10 min blocks for 30 min. The foot and toes were visually monitored to ensure there was no significant motor activity. TMS input output curves were obtained prior to and every 10 min during stimulation and for 30 min following stimulation.

4.2.6 Simultaneous muscle and cutaneous afferent stimulation

Muscle and cutaneous afferents were concurrently activated in a group of 4 subjects by combining muscle tendon vibration and superficial peroneal nerve stimulation during rest (described above). An auditory signal was provided to assist the experimenter in matching muscle vibration to superficial peroneal nerve stimulation. Muscular/cutaneous stimulation was performed in 10 min blocks for 30 min. TMS input-output curves were obtained prior to and every 10 min during stimulation and for 30 min following stimulation.

4.2.7 Analysis

AxoScope files containing multiple EMG and ankle flexion force traces were imported to Matlab 5 (The Mathworks, Inc., Natick MA) for offline analysis. Custom Matlab software was used to obtain peak-to-peak and mean rectified values for EMG waves of interest: M-waves during CP stimulation, and MEPs during TMS. The mean rectified values generally showed similar changes to the peak-to-peak values, so only the latter will be shown here. A 40 ms interval preceding the stimulus was recorded and analyzed to obtain background EMG levels. This was done to ensure that subjects maintained background activity at relatively constant levels between repeated measures. Thus, any observed changes in MEP cannot be explained by fluctuating background EMG levels.

The ankle force was recorded and then analyzed at the completion of the experiment. Ankle dorsiflexion force during repetitive CP stimulation was recorded every 2 min during each 10 min stimulation period. Ten stimulation trains were averaged from each recording period. The force generated at each intensity during a MEP input-output curve was determined by averaging 4 responses, yielding a force input-output curve.

MEP input-output curves were examined to determine which stimulus intensity initially produced approximately a half-maximum MEP (MEP_h). When averaging data this TMS intensity was used to analyze EMG and force throughout the experiment. All recorded responses were averaged and analyzed before and after being normalized as a proportion of the initial value before stimulation. Since the TMS intensity ranges varied

between subjects, no one intensity was compared between subjects. Rather, intensities corresponding to values such as the MEP_h or MEP_{max} were compared across subjects.

To facilitate this comparison, the data were fitted with a sigmoid curve of the form:

$y = b + (m - b)/(1 + exp((h - x)/w)$

where y is the MEP, b is the background activity in the absence of a TMS stimulus of x % of maximum stimulator output, m is the maximum MEP, h is the stimulus level producing a half-maximum MEP and w is a measure of the width of the curve. This sigmoid curve generally gave a good fit to the experimental data using a nonlinear least-mean squares method (Fig. 4-3) and is identical to the Boltzmann distribution of Capaday et al. (1999) except for the inclusion of a term b. Since the subjects were contracting voluntarily, there will be a measurable peak-to-peak or mean rectified value of EMG over the period of measurement for the MEP, even if no MEP is evoked. The parameter b captures this background activity in the absence of an MEP.

Repeated measures ANOVAs were performed on unnormalized data for MEP, silent period, and force using within-subjects contrasts to determine the effects of stimulation time on these parameters. Silent period was defined as the period lacking observable EMG activity directly following the MEP and continuing until the recovery of background activity (Garvey et al., 2001) in 2 of 4 superimposed traces (see Fig. 4-4). MEP and force were also normalized as proportions of initial values. This normalization process arbitrarily sets all initial recordings to 1; thus there appears to be no variance within the population initially. Differences from 1 were analyzed using a one-sample, Students t-test.

4.3 Results

4.3.1 Effects of repetitive CP stimulation on the MEP

Both peak-to-peak and mean rectified values were obtained for MEP. Since both modes of analysis yielded similar results, only peak-to-peak values are presented. The TA MEP was quite variable between subjects, probably due to factors that include skin thickness and adiposity. Each subject's baseline TMS input-output curve was examined to determine which TMS intensity evoked a half-maximum MEP (MEP_h) and maximum MEP (MEP_{max}). The stimulus intensities corresponding to these values were compared across experimental times. Average, peak-to-peak values at a TMS intensity corresponding to MEP_h ranged from 0.3-4.9 mV (2.6±1.9 mV; mean ± SD) before and 0.3-10.4 mV (3.8±3.2 mV) after CP nerve stimulation. Most subjects studied showed a reproducible MEP before (Fig. 4-1A) and after (Fig 4-1B) stimulation. The first TA MEP deflection tended to occur at a latency of approximately 30-40 ms following TMS. The latency of the MEP was relatively stable for each subject, remaining constant over a range of TMS intensities and at different experimental times. At low TMS intensities, MEPs appeared as oligophasic deflections. As stimulus intensity increased, MEP amplitude increased and became more polyphasic.

Thirty minutes of repetitive electrical stimulation of the CP nerve at 25 Hz at an intensity capable of evoking 50% M_{max} significantly increased the MEP_h in the TA muscle (repeated measures ANOVA, n=10 subjects, $P<0.05$, unnormalized data). For each measurement subjects maintained a facilitating contraction of 15% MVC. Background EMG levels were monitored for all subjects across all experimental times

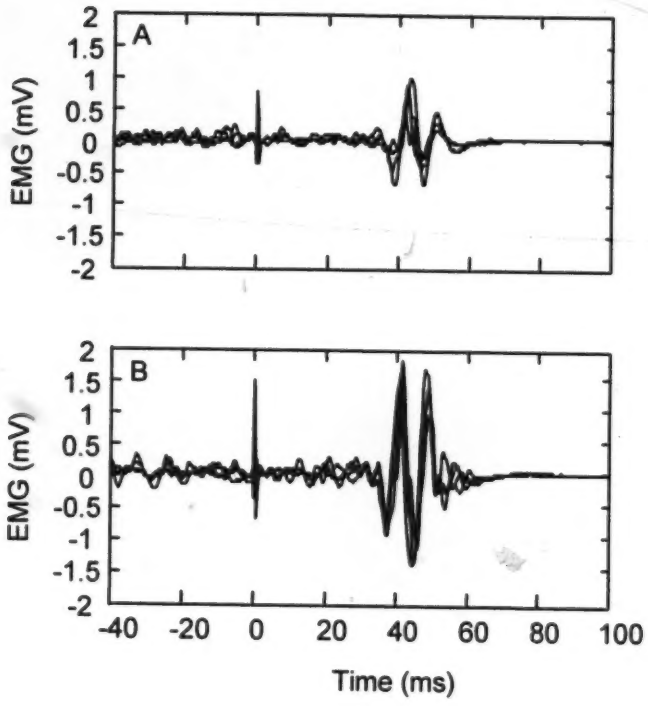

Fig. 4-1 Methods for analyzing the changes in MEP as a result of stimulation. Four superimposed EMG responses of a representative subject to TMS before (A) and after (B) CP nerve stimulation. Note the increased amplitude without a change in latency. Background activity (15% of MVC) was similar between recordings of all subjects (one-sample t-test, $P>0.5$) and a silent period followed the responses.

and showed no significant fluctuations (unnormalized data, repeated measures ANOVA, $P>0.35$; normalized data, one-sample t-test, $P>0.5$). Augmentation of the MEP_h occurred as early as 10 min after the beginning of stimulation and remained elevated for the remainder of the study (Fig. 4-2A). All points after 10 min of stimulation except that at 20 min are significantly above the initial value and none of the points are significantly different from one another. Although the background EMG activity remained constant, the MEP_h increased, due to a leftward shift of the input-output curve (see below and Fig. 4-3A)

At the end of 30 min of stimulation 9 of 10 subjects showed an increase in MEP_h (50.3±13.6%, mean ± SE; n=10, range -4 to +115%). To place all subjects on the same scale, data were normalized with respect to the initial pre-stimulation values. This normalization greatly reduced the variance within the data. Average MEP_h values were significantly elevated at all times following 10 min of CP stimulation, when normalized to the initial value (one-sample t-test, $P<0.05$; Fig. 4-2B). After recording the MEP we measured M_{max} which was determined from the M-wave input-output curve, normalized as a proportion of the initial M_{max} before stimulation and plotted on the same scale as the normalized MEP_h (Fig. 4-2B). In contrast to the MEP_h, M_{max} decreased in 8 of 10 subjects during stimulation (decrease at 30 min was 18.1±5.3%; mean ± SE). Two subjects showed no significant change of M_{max}. Normalized MEP_h and M_{max} changed in opposite directions (paired t-test, $P<0.0001$). There was some recovery towards initial values during the 30 min period following repetitive CP stimulation but neither measure returned fully to its initial values.

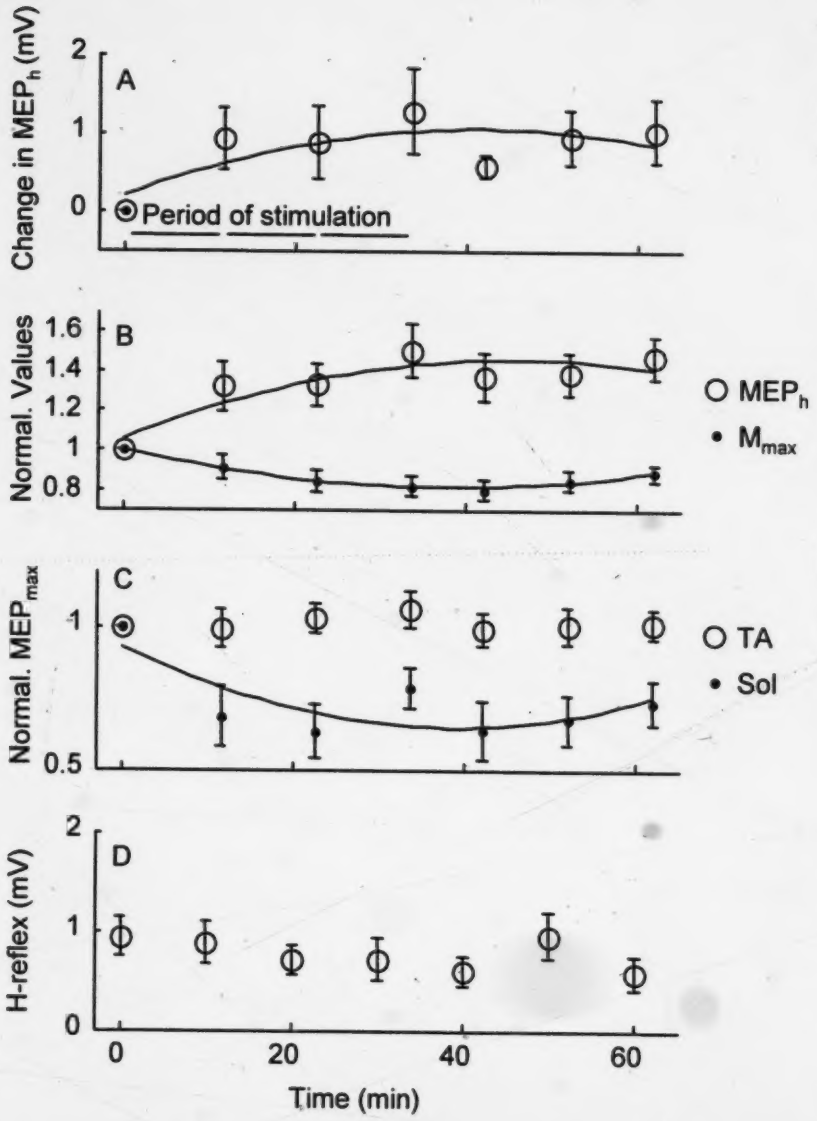

Fig. 4-2 Changes in MEP, M-wave and H-reflex as a result of 30 min of CP stimulation. A: The half-maximum value (MEP_h) increased after 10 min of stimulation and remained increased throughout the period of study. Initial values were subtracted from the average MEP_h (mV) for each subject. B: Average normalized MEP_h increased while that for M_{max} decreased, so the augmentation was not due to muscle properties. C: Normalized TA MEP_{max} did not increase significantly at any time. Normalized soleus MEP_{max} (shown here) decreased following CP stimulation as did soleus MEP_h. D: the H-reflex amplitude did not increase with stimulation time. Data are means ± SE. Parabolas were computed by the least mean squares method to show the trends in A, B, and C that were significant.

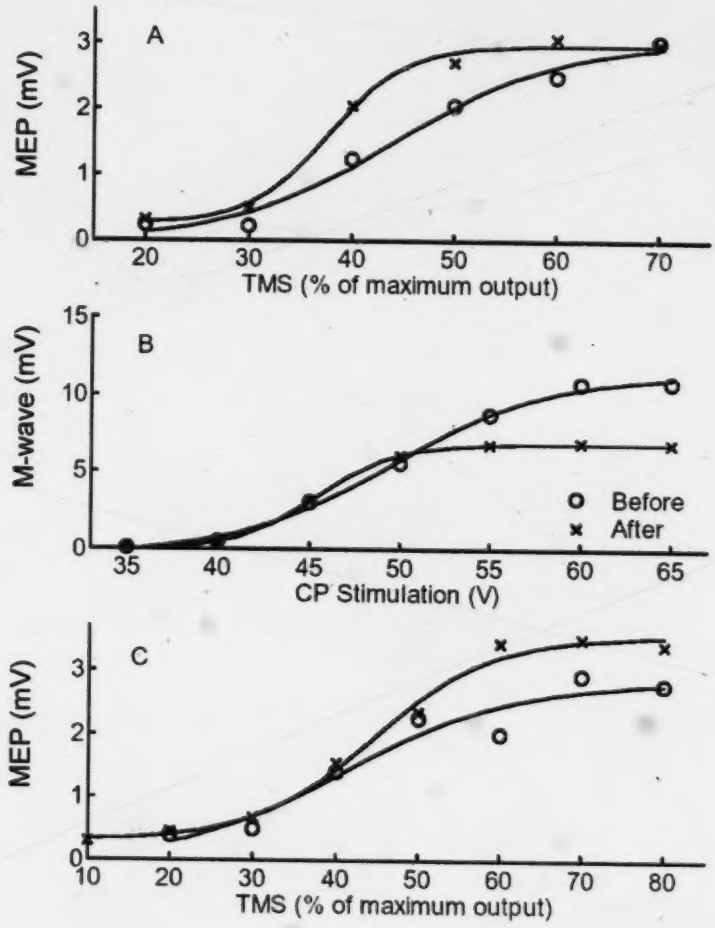

Fig. 4-3 Input output curves for MEP and M-waves. A: MEP input-output curves directly before (O) and immediately after (×) 30 min of stimulating the CP nerve. The peak remains the same, but the curve shifts to the left. B: Maximum M-waves during the same stimulation showed a decrease near the peak. C: MEP before and after 30 min of stimulating the superficial peroneal nerve. The increase mainly occurred near the peak. Sigmoid curves of the form given in the Methods were fitted to each data set with correlations $r^2 > 0.94$. Displayed data from representative subjects.

To check if the augmentation of TA MEP was due to a non-specific global increase in excitability, soleus MEP was measured in four subjects. Soleus MEP amplitudes were normalized to a proportion of pre-stimulation baseline values. Soleus MEP_h was not facilitated at any recording time (one-sample t-test, $P>0.05$). Soleus MEP_{max} was decreased at all experimental times compared to baseline, showing significant changes at 20, 40, 50, and 60 min (one-sample t-test, $P<0.05$) (Fig. 4-2C). TMS values were also obtained in subjects at rest in the TA. Although resting MEP followed a trend similar to facilitated MEP (i.e. increased following CP stimulation) several limitations compromised the reliability of this technique. Firstly, MEP responses at rest were highly variable within subjects even at a single recording time. Secondly, some subjects did not have clear, reproducible MEPs before CP nerve stimulation, preventing a reliable set of baseline values. Despite these limitations, resting TA MEP was significantly facilitated at 40, 50, and 60 min (paired t-test, $P<0.05$, n=10).

The values of MEP max were also normalized as a proportion of the initial value for each subject and averaged. Although the MEP_h showed significant and reproducible increases following CP stimulation, the MEP_{max} did not change significantly (Fig. 4-2C). This is consistent with a leftward shift of the input-output curve, as will be discussed below.

4.3.2 Effects of tendon vibration on TA MEP

Thirty min of tendon vibration at rest produced no significant facilitation of the TA MEP at any experimental time (one-sample t-test, $P>0.05$). MEP input-output curves

from all experimental times were essentially superimposable (not shown), suggesting that there was no enhancement of excitability of the corticospinal tract.

4.3.3 Effects of superficial peroneal stimulation on TA MEP

The MEP input-output curves obtained before and after stimulation of the superficial peroneal nerve differed mostly at the plateau (Fig. 4-3C). MEP_{max} was significantly facilitated at the end of stimulation (31.6±17.0%, mean ± SD; one-sample t-test, $P<0.05$). In contrast, MEP_h was only significantly facilitated at 50 min (one-sample t-test, $P<0.05$) with stimulation of the superficial peroneal nerve. Although these results are interesting, the pattern of facilitation is different from that with CP nerve stimulation (i.e. increase in MEP_h at all times, but not MEP_{max}).

4.3.4 Effects of combined tendon vibration and superficial peroneal nerve stimulation on TA MEP

Simultaneous vibration of the TA tendon and electrical stimulation of the superficial peroneal nerve had no statistically significant effect on any parameters of the TA MEP input-output curve. This stimulation protocol failed to reproduce the facilitation of MEP_h associated with CP nerve stimulation (one-sample t-test, $P>0.05$) or the facilitation of MEP_{max} associated with superficial peroneal nerve stimulation alone (one-sample t-test, $P>0.05$).

4.3.5 Effects of repetitive CP stimulation on H-reflexes

TA H-reflexes could only be elicited in five of the subjects studied. H-reflexes appeared as small-amplitude, oligophasic deflections approximately 35-45 ms following a single electric pulse delivered to the CP nerve, compared to a latency of 8-12 ms for M-waves. CP nerve stimulation activates Ia afferent axons that project to the spinal cord and synapse on motoneurones. The resultant excitation leads to the H-reflex on the EMG. Fig.4-2D shows that the H-reflex amplitude showed no significant increase at any recording time (unnormalized data, unpaired t-test, $P>0.05$; normalized data, one-sample t-test, $P>0.05$). Data from each subject were analyzed using an unpaired t-test. No individual showed a facilitated H-reflex at the end of stimulation.

4.3.6 Effects of repetitive CP stimulation on silent period

Fig. 4-4 shows superimposed responses to TMS with a fixed intensity at various times during and after stimulation. Following the MEP there is a silent period (SP) in EMG activity, although subjects attempted to maintain a constant contraction level. The SP increased in duration with increasing intensities of TMS. At all TMS intensities the latency from the TMS pulse to the beginning of the SP was relatively constant within subjects. Since some portion of the SP is supposed to be of cortical origin, a changing SP duration following CP stimulation may suggest the involvement of cortical processes.

The duration of the silent period at MEP_h was measured for four trials at each time point for each subject and values for all subjects (n=10) were averaged together. Data were not normalized as silent periods in all subjects were within an order of magnitude at MEP_h (range, 31.2-102.9 ms, 60.8±33.5 ms; mean ± SD). Only one subject

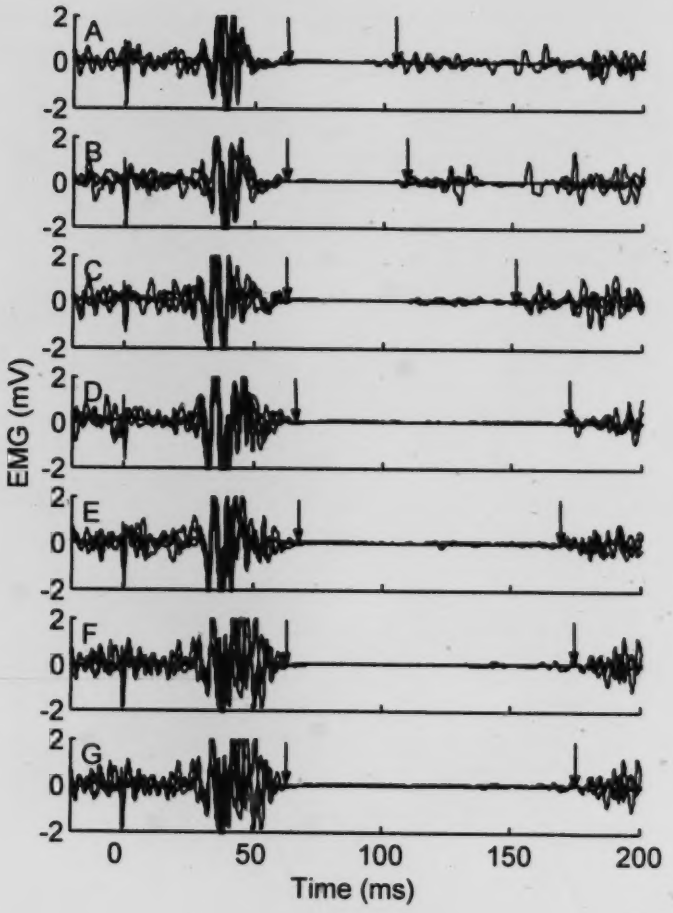

Fig. 4-4 The silent period in the MEP increased with repetitive CP nerve stimulation The silent period (SP) is illustrated for a representative subject at 0 (A), 10 (B), 20 (C), 30 (D), 42 (E), 52 (F), and 62 (G)min after the start of stimulation. Each part contains four superimposed traces at an intensity that initially produced MEP_h. The TMS artifact occurs at 0 ms; the MEP has a latency of about 30 ms and the SP begins at about 65 ms (first triangle). The SP duration (up to the second triangle in each part) increases and shows no evidence of returning after stimulation.

showed a shortened SP at the end of stimulation (87.5% of the initial value, at a TMS intensity of MEP_h). The SP always began at about the same latency following the TMS pulse and was extended in all subjects at all recording times during the 30 min following stimulation. The duration of the SP continued to increase on average even after the end of repetitive CP stimulation (Fig. 4-5A), although the changes were not significant. On average the SP duration at MEP_h was prolonged by 75.3±12.9% (mean ± SE) at 62 min (99.3±14.8ms as measured from end of MEP). SP duration is positively correlated to MEP amplitude. To determine if SP and MEP increased in parallel, the ratio of SP: MEP was calculated (Fig. 4-5B). SP was significantly increased by a greater proportion than MEP 10 and 20 min following repetitive CP nerve stimulation (one-sample t-test, $P<0.05$).

4.3.7 Effects of repetitive CP stimulation on ankle flexion

The force each subject exerted on the footplate in response to a single TMS pulse varied greatly, ranging from 3.5-29.4 Newtons (13.3±10.2 N; mean ± SD) initially and 4.0-80.2 N (28.3±25.7 N) at the end of stimulation. Data were analyzed in response to TMS pulses given at MEP_h and normalized as a proportion of each subject's initial value. Force increased during repetitive CP stimulation, attaining a maximal increase immediately after stimulation (Fig. 4-6). One subject showed no significant facilitation of force at the end of stimulation. The force in the 9 responding subjects ranged from 166-320% of initial values after 30 min of repetitive CP stimulation (203±73 % mean ± SD, n=10). Force did not immediately decrease at the termination of stimulation but remained

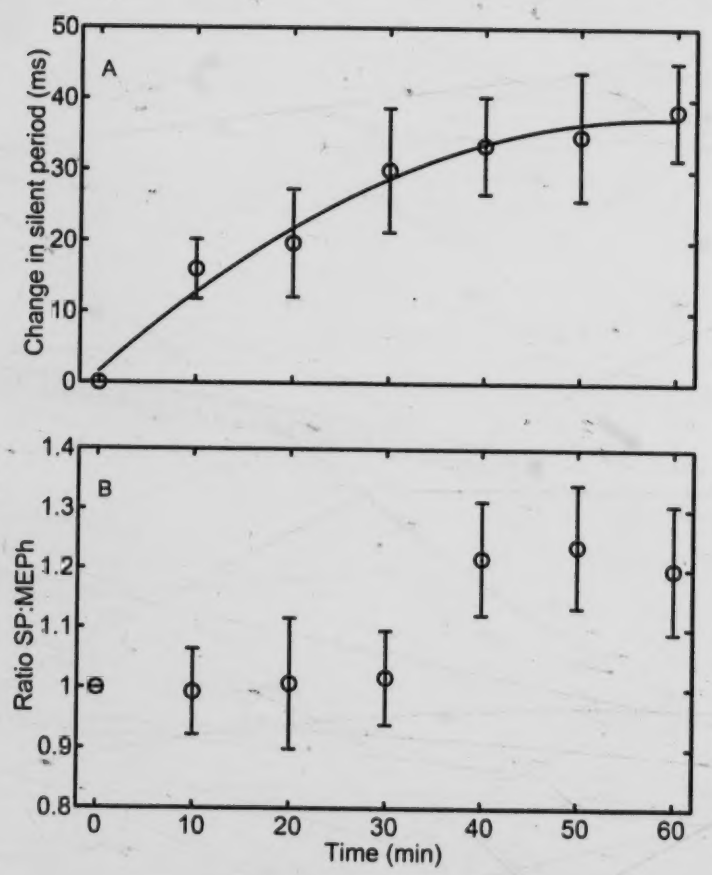

Fig. 4-5 Average increase in SP duration. A: The SP duration increased at 10 min of stimulation and continued to increase for the duration of the experiment. Measurements taken at all times were significantly increased (repeated measures ANOVA, $P<0.05$). Data points are averages (n=10 subjects) with standard error bars. The data can be fit with a parabola (solid line) which shows the asymptotic increase of the SP ($r^2=0.98$). B: Ratio of normalized SP to MEP_h demonstrates SP duration does not merely increase linearly with MEP amplitude. The ratio is significantly above 1 at 40 and 50 min (one-sample t-test, $P<0.05$).

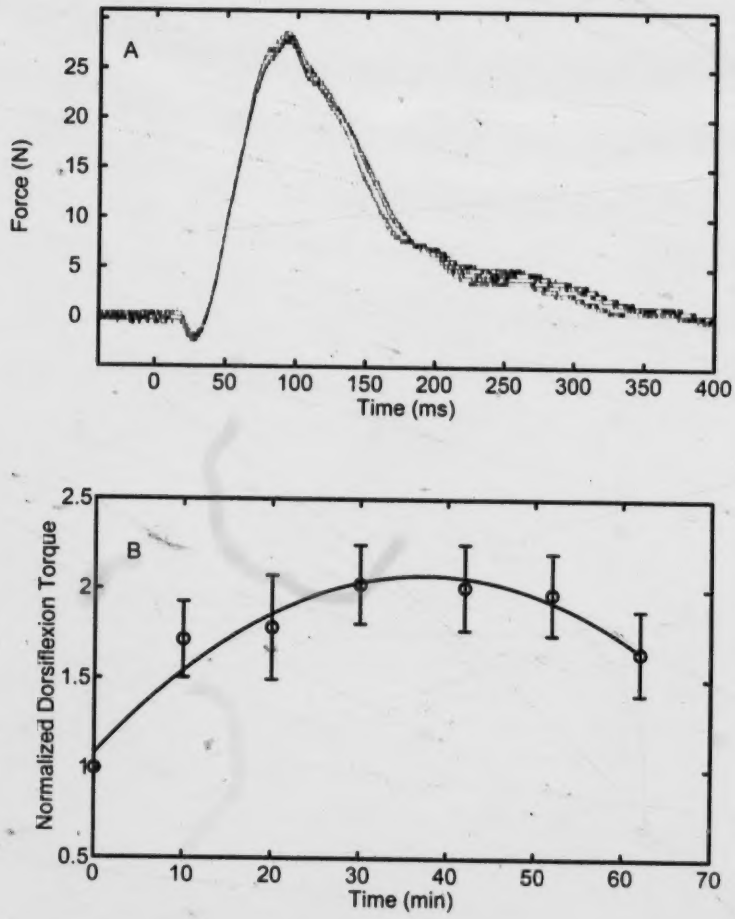

Fig. 4-6 Increase in force with stimulation of the CP nerve. A: Four superimposed force traces obtained at a TMS intensity corresponding to MEP_h. Peak-to-peak force was measured from the baseline (pre-deflection) to the upper peak. B: Average (n=10 subjects) dorsiflexion force of the ankle normalized with respect to initial values in response to TMS at an intensity that initially gave MEP_h. All time points are significantly increased compared to initial values (one sample t-test, $P<0.05$). The force increased at 10 min and showed a 102±21.9% (mean ± SE) enhancement at 30 min. It remained elevated, only showing an insignificant decrease at 62 min (65% enhancement). The data are again fitted with a parabola to show the trend.

elevated at 42 and 52 min recording times. Only at 62 min did force decline, but the decrease was not statistically significant compared to 52 min.

4.4 Discussion

4.4.1 Repetitive CP stimulation facilitates the MEP

The increase in the MEP in the TA muscle suggests that stimulation does more than simply activate the muscle; it also facilitates corticospinal projections over the short term. The changes lasted for at least 30 min following stimulation without decrement. Others have demonstrated similar short-term changes in the CNS (Ziemann et al., 2001; Ridding et al., 2001; Khaslavskaia et al., 2002). The significant increase in MEP occurred at MEP_h rather than at the maximum level, consistent with a leftward shift of the input-output curve. Thus, existing corticospinal connections are easier to activate, suggesting that the facilitation of the MEP is due to increased excitability, rather than total connectivity. Since this facilitation outlasts the stimulation, gait in patients with a weakness in this muscle may be improved even after stimulation is discontinued. An interesting result obtained in the present study is that during facilitation of the MEP_h, peak-to-peak M_{max} concurrently decreases. The maximum electrical activity in the TA muscle is reduced, indicating that MEP potentiation does not occur at the level of the muscle fibre or neuromuscular junction as potentiation at one of these distal levels would result in an increased MEP and M_{max}.

Subjects for whom the soleus MEP was measured showed consistent decreases in MEP_h and MEP_{max} during concurrent enhancement of TA MEP_h. This suggests that repetitive CP nerve stimulation elicits specific facilitation of the TA muscle rather than a

non-specific global enhancement of excitability. The increased activity in TA may account for the diminished soleus MEP via recurrent inhibitory pathways. Clinically, inhibiting soleus may enhance the benefits afforded by repetitive CP stimulation to patients suffering from foot drop.

4.4.2 Facilitation is not due to excitability changes at the level of motoneurones

A potential site of MEP facilitation is the spinal motoneurone. If the soma or axon hillock of the motoneurone became more excitable, H-reflex amplitude should increase in parallel with the MEP. An unchanging H-reflex throughout the duration of the experiment suggests there is no change in motoneuronal excitability. This is consistent with a supraspinal or interneuronal site for MEP facilitation. Khaslavskaia et al. (2002) used 200 Hz stimulation for 30 min to elicit a decrease in threshold for the MEP input-output curve. They observed no facilitation of the TA short-latency stretch reflex, indicating that their effect was also not due to increased excitability of the motoneurones. Since the H-reflex method activates motoneurones relatively directly and the short-latency stretch reflex activates more physiologically relevant pathways (including interneurons), our results taken with those of Khaslavskaia favor a supraspinal site of modulation. However, the populations of motoneurones activated by TMS and the H-reflex technique may not perfectly overlap. Thus H-reflex data may not be completely comparable to TMS.

4.4.3 Facilitation is not similar to the effects of fatigue

A body of literature suggests that fatigue due to a voluntary contraction can facilitate MEP in a number of muscles, but the effects occur over a different time course than found here (Lentz and Nielson, 2002; Zijdewint et al., 2000; Samii et al., 1996). During fatigue MEP is elevated for a very short time, returning to baseline within seconds and falling to lower values for as long as 30 min (Taylor and Gandevia, 2001). Fatigue studies have also shown that the M-wave briefly increases and returns to the initial value at 2-3 min (Lentz and Nielson, 2002), again opposite to the results in the present study. Pitcher and Miles (2002) compared voluntarily induced fatigue to electrically induced fatigue in the first dorsal interosseus (FDI) muscle. Whereas voluntarily induced fatigue depressed the FDI MEP for at least 20 min, electrically induced fatigue facilitated the FDI MEP for approximately 10 min. By 20 min the MEP in the electrical condition fell and remained depressed for at least 60 min following fatigue. The MEP response profile measured in the present study does not match that of voluntarily or electrically induced fatigue. MEP increased during stimulation and peaked at 30 min, but remained elevated for the rest of the study time. Three subjects received 30 min of repetitive CP stimulation, but were followed for 90 min after stimulation, instead of 30 min. At this extended time MEPh had decreased towards, but not below initial values.

4.4.4 Facilitation is consistent with corticospinal changes in excitability

The significant increase in TA MEP occurred at MEP_h rather than MEP_{max}. The slope of an input-output curve represents the gain of corticospinal pathways (Devanne et al., 1997). An increase at MEP_h without an associated facilitation of MEP_{max} corresponds to an increase in excitability of descending projections. Repetitive stimulation of the ulnar

nerve (a mixed nerve like CP) causes a change in the excitability of the primary motor cortex in healthy humans. However, no changes in excitability are evident in response to transcranial electrical stimulation, which activates the corticospinal tract subcortically, suggesting the plasticity occurs at the cortical level (Ridding et al., 2000). Facilitation only occurred in muscles innervated by the ulnar nerve. Thus, the effect is thought to be a specific, rather than a global increase in cortical excitability. If connections to extensors were potentiated by CP stimulation to the same extent as those to flexors (i.e., soleus as well as TA), facilitated plantarflexion would oppose facilitated dorsiflexion and no potentiation of ankle flexion would be observed, in contrast to our findings. If the connections to these muscles were facilitated, it would attenuate the increase in flexor force. In fact, MEP amplitudes in soleus were reduced (Fig. 4-2), suggesting that any increases are specific to the muscles stimulated. Also, Khaslavskaia et al., (2002) showed no accompanying change in soleus MEP during TA MEP facilitation.

In a study analogous to the present work Hamdy et al. (1998) gave 10 min of 10 Hz sensory stimulation to the pharyngeal region via swallowed electrodes. After stimulation pharyngeal MEP amplitudes were significantly increased for up to 30 min while esophageal MEP amplitudes were either unaffected or decreased. By using TMS to map swallowing regions of the cortex they found the cortical representation of the pharyngeal region increased and the esophageal region decreased, consistent with specific, rather than global changes.

TMS results in D (due to Direct activation of pyramidal tract neurons) and I (due to Indirect activation of pyramidal tract neurons via interneurons) descending volleys (Awiszuz and Feistner, 1994). I waves are generally elicited at a lower stimulus intensity

than D waves (Di Lazzaro et al., 2001). In the subjects studied, there was no change in MEP latency between low and high stimulus intensities, suggesting we were not consistently evoking D waves at higher intensities. Also, D waves would have a larger influence on peak-to-peak MEP compared to mean rectified MEP. Since both forms of MEP analysis revealed the same trends, it is unlikely that D waves dominated the MEP amplitude.

4.4.5 Cortical effects and the silent period

In healthy individuals a silent period follows the MEP in the EMG of contracting muscles. As MEP magnitude increases, SP duration tends to increase (Trompetto et al., 2001). The SP is partly due to both spinal mechanisms and intracortical inhibition (Ziemann et al., 1993). The excitability of spinal motoneurones, evaluated by eliciting H-reflexes, is severely depressed at the beginning of the SP, but recovers towards the end of the SP. Thus, the early phase is due to spinal mechanisms while the late portion is probably due to cortical structures (Fuhr et al., 1991). Other studies suggest that the cortical contribution to the SP greatly outweighs spinal effects (Bertasi et al., 2000). Shimizu et al. (2000) described patients who were lacking a SP after sustaining cervical spinal cord lesions, suggesting a supraspinal mediator. Schnitzler and Benecke (1994) presented two representative patients who suffered focal ischemic lesions localized to the primary motor cortex. The patients were lacking both early and late phases of the SP on the side contralateral to the lesion, while a normal SP was observed in the ipsilateral muscles, again emphasizing the importance of cortical structures. More recent papers also

support the theory that the inhibitory mechanism lies within the primary sensorimotor cortical region, as opposed to non-primary motor areas (Ikeda et al., 2000).

On the assumption that the late phase of SP is largely a cortical phenomenon, it was evaluated to determine if CP stimulation had any cortical effects. The SP became very long in response to repetitive CP stimulation (Fig. 4-4; >100 ms), possibly suggesting supraspinal effects. Various methods for measuring SP duration exist, such as setting the beginning of SP to the stimulus artifact (Tinazzi et al., 2003). The method employed in the present study (defining SP onset as MEP termination) results in substantially shorter SP values than if the measurements were from the stimulus artifact. Although MEP amplitude and SP duration are positively correlated, SP increased by a greater proportion than MEP (Fig. 4-5B). This suggests that not all of the SP increase can be accounted for by MEP facilitation. Afferent projection to the primary sensorimotor area is a possible mechanism for cortical effects from stimulation. Since the late phase of the SP is likely a cortical phenomenon, the changes in MEP may be due to cortical events. The apparently anomalous finding of mutually enhanced MEP and silent period (related to intracortical inhibition) is consistent with a concurrent facilitation of multiple excitatory and inhibitory neuronal sub-populations within a localized region of cortex, as opposed to facilitation of an individual cell type. Previous work has suggested that rapid plasticity of the motor cortex may be due to modulation of GABAergic inhibition and activity of N-methyl-D-aspartate receptors (Bütefisch et al., 2000; Ziemann et al., 1998).

4.4.6 Lack of effects from activation of muscle afferents

We could not demonstrate any significant lasting facilitation of MEP or force following tendon vibration while subjects were at rest. Apparently, muscle afferent input alone is insufficient to induce cortical plasticity. Stefan et al. (2002) showed that repetitive application of single afferent electric stimuli to the median nerve, paired with TMS at a latency capable of producing synchronous events in the primary motor cortex, elicited a rapidly evolving and long-lasting increase in corticomuscular excitability. They argued that afferent input induces cortical disinhibition, presenting a more excitable substrate for TMS to act upon. Possibly, simultaneous afferent and motor activity results in coupling, mediating the observed facilitation of MEP. Additional evidence for the importance of paired motor and afferent activity was presented by Sinkjaer and Khaslavskaia in an abstract (2002). Subjects who received repetitive CP nerve stimulation while at rest showed a MEP potentiation of 26% whereas subjects who received the same stimulation while voluntarily contracting the dorsiflexors showed a potentiation of 142%.

4.4.7 Effects of preferential activation of cutaneous afferents

Although stimulation of the superficial peroneal nerve resulted in MEP facilitation, the facilitation pattern did not match that obtained from the CP nerve stimulation. This suggests that cutaneous afferents alone are not responsible for the shift we observed in the MEP input-output curve. The observed increase of MEP_{max} following superficial peroneal nerve stimulation confirms the results of Khaslavskaia et al. (2002) who showed a similar increase of MEP_{max} following a 200 Hz afferent dominated stimulation protocol. We doubled the stimulation frequency of the CP nerve for the

superficial peroneal nerve in an attempt to account for the smaller number of cutaneous fibres passing through the latter nerve. In using such high frequencies we may have inadvertently induced frequency dependent LTP-like processes within the CNS not active during lower frequency CP nerve stimulation. Possibly, co-activation of muscle and cutaneous afferents, or coupling with motor output is necessary to elicit the MEP_h facilitation produced during repetitive CP nerve stimulation. We could not determine the mechanism underlying this phenomenon unequivocally employing the available experimental techniques.

4.4.8 Lack of effects of tendon vibration and superficial peroneal nerve stimulation

No significant facilitation of TA MEP was observed during coincident TA tendon vibration and superficial peroneal stimulation. This suggests that activation of muscle and cutaneous afferents alone is unable to mimic the facilitation of MEP_h during CP nerve stimulation. We were unable to reproduce the results obtained during CP nerve stimulation by dissociating afferent inputs. Unfortunately, we are unable to test the effects of pairing motor output with afferent input using the current experimental techniques since no purely motor nerves exist without afferents from the innervated muscles in the limbs, and volitional activation of a muscle results in muscle and cutaneous afferent input. Thus, motor activity cannot be isolated as easily as sensory activity.

4.4.9 Stimulation allows subjects to generate more force at a given level of cortical drive

EMG activity and force produced by a muscle are positively correlated (Onishi et al., 2000; Totosy de Zepetnek, 1991). Thus, an increase in force at MEP_h is expected in subjects who show a corresponding increase in MEP. The normalized average curves for MEP and force (Fig. 4-2B and 4-6B respectively) show nearly the same trends. Force was dramatically increased during repetitive CP stimulation and remained significantly increased for the whole period studied. Force may be briefly augmented during fatiguing protocols, but generally falls quickly below initial values. Sacco et al. (1997) showed that twitch force in response to TMS was briefly facilitated at times during a fatiguing contraction of elbow flexors (40-80% of the endurance time). Later, (90-100% of endurance time) the TMS-induced twitch force fell below initial values. Presumably, the larger force in our experiments results from the MEP activating more motoneurones.

4.4.10 Possible Mechanisms

Although the effects of CP stimulation on SP may suggest cortical involvement in the facilitation of TA MEP, the evidence is indirect. The interpretation is complicated by spinal contributions to SP. Had MEP facilitation occurred during sensory stimulation alone, we may have argued more strongly for a cortical mechanism, based on direct afferent projections to the cortex. However, the apparent requisite of concurrent activity in efferent and afferent fibres may suggest a segmental level of plasticity. α-motoneurones send recurrent collaterals to Renshaw cells in the spinal cord which synapse onto Ia inhibitory interneurones and antagonistic α -motoneurones (Windhorst, 1996). Inhibition of antagonistic α -motoneurones may in turn disinhibit the motoneurones of interest. Also, the Ia interneuron serves as a point of convergence

between efferent (Renshaw cells) and afferent (Ia muscle spindle afferents) signals and may be a point of importance in efferent/afferent-dependent plasticity. Some Renshaw cells inhibit agonist (i.e. TA) α -motoneurones and Ia inhibitory interneurons synapsing on antagonist (i.e. soleus) α -motoneurones (Windhorst, 1996). If this population of Renshaw cells were to become less active, agonist α -motoneurones would be less inhibited whereas antagonist α - motoneurones would be less active due to disinhibited Ia interneurons. This circuit may explain the concurrent facilitaion of TA MEP and decrement of soleus MEP following repetitive CP nerve stimulation. Although we present indirect evidence of cortical effects of CP nerve stimulation we cannot determine the relative importance of cortical changes and polsynaptic changes at the segmental level. If the plasticity described in this study occurred at a polysynaptic interneuronal level, H-reflex facilitation would not be evident. Another possibility is that Renshaw cells or Ia interneurons may somehow transmit efferent information to the cortex, although we know of no such pathways.

4.4.11 Clinical implications

Stimulation of the CP nerve at 25 Hz is clinically interesting; during functional electrical stimulation (FES) patients with foot drop receive this frequency of CP stimulation (Wieler et al., 1999). Foot drop is a condition typified by decreased activity in the TA muscle to dorsiflex the ankle and often increased tone in ankle extensors (Capaday et al., 1999), so facilitatating corticalspinal excitability and ankle flexion in these patients may alleviate foot drop. Although the exact site and detailed mechanism could not be determined, repetitive stimulation of the CP at 25 Hz increased both the

MEP and force in TA muscles of healthy subjects while decreasing MEP in soleus, likely due to short-term CNS plasticity. Although repetitive CP stimulation caused a decrease in M_{max} amplitude, it did not induce muscle weakness. The fact that the demonstrated plasticity occurred during voluntary activity of the TA suggests this sort of plasticity may have functional benefits, contrary to concerns raised by previous work (Ridding and Rothwell, 1995). Further studies on neurological patients are required to determine if these results are clinically relevant. Promising preliminary results have been obtained from 2 patients (males with incomplete spinal cord lesions) who showed similar or even enhanced responses to CP stimulation compared to the control subjects studied here.

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5. SHORT-TERM EFFECTS OF FUNCTIONAL ELECTRICAL STIMULATION ON MOTOR EVOKED POTENTIALS IN ANKLE FLEXOR AND EXTENSOR MUSCLES

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5.1 Introduction

In functional electrical stimulation (FES) some residual pathways below the spinal cord level after the injuries or diseases may be activated to restore lost function. Regular use of FES induces long-term effects, such as increased muscle strength, fatigue resistance, and bone density (Martin et al. 1992; Stein et al. 1992; Belanger et al. 2000; Gerrits et al. 2000), as well as reducing spasticity (Solomonow et al. 1997). Several studies have also reported that walking speed increases substantially with the regular use of FES of the common peroneal (CP) nerve in hemiplegic patients and patients can walk better even without FES (Stein et al. 1993; Burridge et al. 1997; Barbeau et al. 1998; Taylor et al. 1999; Wieler et al. 1999; Ladouceur and Barbeau 2000a; Ladouceur and Barbeau 2000b; Barbeau et al. 2002). These observations suggest that plastic changes take place in the nervous system as a consequence of FES-aided walking, likely due to the effect of regularly applied stimulation, increased locomotor activity (i.e., training effect), or the combination of both. In fact, treadmill training with partial body weight support has been shown to improve locomotor activity in individuals with incomplete and complete spinal cord injury (Wernig and Muller 1992; Dietz et al. 1994; Dietz et al. 1995; Wernig et al. 1995; Dietz et al. 1997; Colombo et al. 1998; Dietz et al. 1998; Wernig et al. 1999; Dietz and Duysens 2000; Wirz et al. 2001; Maegele et al. 2002; Dobkin et al. 2003)

or stroke (Barbeau and Visintin 2003). How locomotor activity and/or FES affects the nervous system, especially descending connections, over the period of FES-aided walking has not been investigated in either a short or long time frame.

Transcranial magnetic stimulation (TMS) and the motor evoked potential (MEP) are available to study corticospinal excitability (Pascual-Leone et al. 2002). The silent period (SP) after an MEP has also been shown to, at least partly, reflect cortical inhibition (Fuhr et al. 1991; Ziemann et al. 1993; Chen et al. 1999; Bertasi et al. 2000; Shimizu et al. 2000; Wu et al. 2000; Weber and Eisen 2002). In parallel to increased corticospinal excitability, reduced intracortical inhibition has an important role in inducing plastic changes in the cortex (Jacobs and Donoghue 1991; Ziemann et al. 1998a; Ziemann et al. 1998b; Ridding and Rothwell 1999; Butefisch et al. 2000; Ziemann et al. 2001; Chen et al. 2002; Kaelin-Lang et al. 2002; Levy et al. 2002).

The MEP in a target muscle elicited by TMS increases when stimulating or altering cutaneous and/or muscle afferents. For instance, transient deafferentation of the hand or forearm leads to increased MEP in muscles proximal to the site of ischemia (Brasil-Neto et al. 1993; Ziemann et al. 1998a; Ziemann et al. 1998b; Werhahn et al. 2002). Repetitive stimulation of peripheral nerve induces reorganization of the motor cortex and an increase of corticospinal excitability (Hamdy et al. 1998; Ridding et al. 2000; Ridding et al. 2001; Kaelin-Lang et al. 2002). In combination with TMS, manipulating afferent activity has also been shown to have lasting effects on cortical excitatory and inhibitory circuits (Ridding and Rothwell 1999; Stefan et al. 2000; Ridding and Taylor 2001; McKay et al. 2002; Stefan et al. 2002; Charlton et al. 2003). Most of these studies have been done in hand or arm muscles and only a few studies have

dealt with leg muscles. Khaslavskaia et al. (2002), Knash et al. (2003), and Sinkjaer and Khaslavskaia (2002) stimulated the CP nerve repetitively for a period of time, and found short lasting (30 min to 1 hr) facilitatory effects on the MEP in the tibialis anterior (TA) muscle. Thus, stimulating peripheral nerve seems to facilitate corticospinal descending connections in both upper and lower limbs.

Voluntarily activating particular muscle groups also induces changes in the motor cortex. Butefisch et al. (2000), Liepert et al. (2000), and Ziemann et al. (2001) reported that short-term practice/training of particular movements of the upper limb increased the MEP elicited in target muscles. Does use of the legs lead to a similar increase of MEP in leg muscles? Thus, both stimulation of sensory and/or muscle afferents and use of a particular part of the body increase corticospinal excitability and induce reorganization of neural circuitries in the CNS, at least for upper limb muscles.

Based on these findings, combination of the stimulation and use might be expected to show even stronger facilitatory effects on the corticospinal excitability and connectivity. In fact, Sinkjaer and Khaslavskaia (2002) stimulated the CP nerve repetitively during voluntary facilitation of the ankle flexors, and reported larger increase in the TA MEP with a combination of nerve stimulation and voluntary facilitation than with stimulation alone. What would happen if the nerve stimulation is applied during a functional movement, such as walking, to assist stepping? Is the stimulation at functionally important times during walking more effective than passively received repetitive peripheral nerve stimulation during sitting in terms of increasing corticospinal excitability? Does walking alone improve the descending connection?

In the present study, electrical stimulation was applied to the CP nerve during the swing phase of the step cycle. This is referred to clinically as functional electrical stimulation (FES), since the stimulation is applied at functionally relevant times in the step cycle. The purpose of this study was to investigate short-term effects of FES during walking on MEPs and SPs in the ankle flexors and extensors. FES was delivered using the WalkAide2 foot drop stimulator (Stein 1998), which activates ankle dorsiflexors during the swing phase of the step cycle. MEPs and SPs in the TA and soleus muscles were measured before, between, and after periods of walking with or without FES, to examine whether the changes were specific to the muscle stimulated or extended to the nearby (even antagonist) muscle. We also treat foot drop in persons who have had a stroke or spinal cord injury (Wieler et al. 1999), so this study is a prelude to examining the long-term effects in patient populations.

5.2 Materials and methods

5.2.1 General procedure

Ten neurologically normal subjects aged from 25 to 46 participated in this study. All subjects gave informed consent for the purposes and procedures of the experiments, as approved by the Human Ethics Committee of the University of Alberta. Two different experiments were performed in each subject on different days. In one experiment the subject walked on a treadmill with the FES device on, and in the other one without FES. The basic experimental condition and procedure were the same for both experiments. The order of two experiments was randomized among subjects.

Measurements were made with the subject comfortably seated in a chair with the right or left foot fixed in a footrest. The hip, knee, and ankle joint angles were approximately 100°, 120°, and 110°, respectively. At the beginning of each experiment, the maximum tonic voluntary contractions (MVC) of the TA and soleus muscles were determined as the maximum rectified EMG level. All the MEP measurements were made in a sitting position. The foot was fixed, so the ankle angle was approximately 100°, while the subject was voluntarily maintaining the activation of a target muscle EMG (see the section *EMG recordings and functional electrical stimulation*). The input-output relation of the corticospinal pathway was obtained for both the TA and soleus by increasing the TMS intensity from below the MEP threshold to the level at which the MEP reached its maximum amplitude. After the first measurement of MEP input-output curves, the subject walked on a treadmill at 4.5 km/h for 10 min either with or without FES of the CP nerve. During walking, the TMS coil was removed from the head and no EMG was recorded. Then, after 10 min of walking, the subject sat in a chair again, and the second set of MEP measurements was made. This routine was repeated 3 times; that is, the subject walked on a treadmill for 30 min in total. After 30 min of walking with or without FES, MEP measurements were made every 10 min for a further 30 min.

One to seven days later, the subject came back to the laboratory to complete the other experimental session. The same procedure was taken for the second day of experiment, except for the use or disuse of FES.

5.2.2 Transcranial magnetic stimulation

Transcranial magnetic stimulation (TMS), used to evaluate the corticospinal excitability, was delivered from an MES-10 Magneto-Electric Stimulator (Cadwell, Kennewick, WA) via custom-made, double-D coils with radii of 8 cm. The coil was held over the scalp such that the induced current flowed in the posterior-anterior direction in the brain. Under slight voluntary activation of the TA muscle (≅15% MVC), the MEP was elicited by TMS with an interval of 3-4 s between stimuli. The optimal TMS location was determined by moving the coil over the scalp from 0 to 2 cm lateral to and –1 to 2 cm posterior to the vertex to find the location at which the lowest stimulus intensity was required to activate the ankle flexor. Although there was some variability among subjects, usually the location was around 0-1 cm posterior and 1 cm lateral to the vertex. The stimulus location and several reference points were marked on the scalp, so that the coil position and angle could be securely maintained by one of the experimenters throughout an experiment. In order to obtain the input-output relation of the corticospinal pathway, the TMS intensity, represented as a percentage of the maximum current that can be discharged into the coil, was increased in steps of 10% until the MEP reached its plateau. Four MEPs were collected at each intensity, and the peak-to-peak amplitudes were plotted against the stimulus intensity in real time by a custom written program in MATLAB (Mathworks Inc., Natick, MA). Similarly, in 8 subjects the soleus MEP was measured during ≅15% MVC of tonic voluntary contraction at the optimal TMS location for the soleus activation, and the input-output relation was also evaluated. In the present study, all the MEP measurements were made with a constant voluntary activation of the target muscle, so that the level of motoneuronal excitability was held fixed over the

experimental period and changes in corticospinal excitability could be evaluated in a functional state (Ridding and Rothwell 1995; Knash et al. 2003).

5.2.3 EMG recordings and functional electrical stimulation

The soleus and TA EMG signals were obtained using surface self-adhesive Ag-AgCl electrodes (Kendall LTP, Chicopee, MA). EMG recording electrodes were placed 2-3 cm below the gastrocnemius in line with the Achilles tendon for the soleus, and over the motor point for the TA, with ~2 cm interelectrode spacing. The signals were amplified, high-pass filtered at 30 Hz, low-pass filtered at 1 kHz, and recorded with AxoScope (Axon Instrument, Union City, CA) at a sample rate of 5 kHz. EMG signals were also rectified, low-pass filtered (RC filter, break point = 3 Hz), and sent to an oscilloscope, so that subjects could monitor their EMG activity levels during experiments. EMG in response to each test stimulus signal was recorded for 500 ms, including a prestimulus period of 50 ms, so that the SP and background EMG amplitude could be evaluated off-line.

During walking the electrical stimulation was applied to the CP nerve by WalkAide2 (Stein 1998), a single channel FES device that has been developed to prevent foot drop by stimulating ankle flexors during the swing phase of walking for patients with stroke, incomplete spinal cord injury or other CNS disorders. The WalkAide 2 is triggered by a tilt sensor (Stein 1998), and stimulates the common peroneal nerve near the head of the fibula at 25 Hz. Stimulation begins when the leg is tilted backwards at the end of stance and ends when the leg is tilted forward at the end of swing. The stimulus intensity was adjusted to produce approximately 50% of the maximum M-wave for each

subject. Regardless of whether or not FES was applied, the subject was instructed to walk normally. We did not observe any obvious changes in the walking pattern (e.g., stride frequency and difference between the stimulated and unstimulated legs) with FES. No subjects reported fear or danger of tripping or falling during FES-assisted walking.

5.2.4 Data analysis

EMG data, stored as AxoScope files, were analyzed offline using custom written programs in MATLAB (The Mathworks, Inc., Natick MA). Peak-to-peak amplitudes of MEPs were averaged at each intensity and time for each subject, and used to calculate an input-output curve. From the initial input-output curve, a stimulus intensity that produced approximately a half-maximum MEP (MEP_h) was determined for each subject. Note that the initial MEP_h was often not exactly half of the maximum MEP (MEP_{max}), since the TMS intensity was increased by 10% and the intensity for the MEP_h was the closest to the estimated h described below. We used MEPs evoked at this TMS intensity (i.e., MEP_h) for statistical analyses, since they would likely be sensitive to changes in both threshold and slope of the input-output curve. Also, the TMS intensity ranges varied among subjects. Therefore, amplitudes of the MEP_h and MEP_{max}, elicited at the same relative intensities across subjects, were compared in this study. To facilitate this comparison, the data were fitted with a sigmoid curve of the form:

$$y = b + (m - b)/(1 + \exp((h - x)/w)$$

where y is the MEP, b is the background activity in the absence of a TMS of x % of maximum stimulator output, m is the maximum MEP, h is the stimulus level producing a half-maximum MEP and w is a measure of the width of the curve (Devanne et al. 1997;

Knash et al. 2003). The TA and soleus MEP input-output relations were measured before, between, and after walking with or without FES for 30 min. For comparison between different conditions across subjects, MEPs were normalized to the initial values, and analyzed statistically. Comparison with the initial, normalized value was achieved by testing a difference from 1, using a one-sample Student's t-test. Note that in this paper data of mean rectified MEPs are not presented to avoid redundant description in *Results*, since changes in mean rectified MEPs were similar to those in peak-to-peak values. In addition, the background EMG activity was obtained from the mean level of rectified EMG amplitude calculated for a 50 ms of prestimulus period for each EMG response. All the background EMG levels were analyzed to ensure that the background activity level remained unchanged throughout an experiment. The SP was also measured as the period from the end of the MEP to the end of the SP for the TA MEP_h and MEP_{max}. The end of the SP was defined as the recovery of background activity in two of four responses (Garvey et al. 2001; Knash et al. 2003). In order to assess the effect of time, repeated measures ANOVAs were performed on SPs.

5.3 Results

5.3.1 Changes in the TA MEP

MEPs were elicited in the TA during 15% MVC tonic ankle dorsiflexion before, between, and after periods of walking on a treadmill either with or without FES. The background EMG level monitored during the MEP measurement did not change over the experimental period (one-way repeated measures ANOVAs, $P>0.05$ at all TMS intensities). Changes in the TA MEP after 30 min of walking with or without FES at

different TMS intensities are presented in a single subject in Fig. 5-1. Immediately after walking with FES (B), the MEP_h (at TMS 60%) was increased by 40% and 10 min after (C) by 58%, compared to the amplitude before walking (A). MEPs evoked at the TMS output of 50% also increased after walking, and there were measurable MEPs 10 min after at TMS 40%, where none were observed earlier, suggesting a decrease in the threshold. However, measuring the threshold accurately in the presence of voluntary activity is difficult, so we will not consider changes in threshold further. Note that increased MEPs became even clearer 10 min after walking with FES than immediately after. In contrast to the observation in A-C, the TA MEP did not increase with walking alone and sometimes decreased (compare E and F to D).

Fig. 5-2 shows averaged MEP input-output curves in the TA during slight voluntary facilitation for the same subject. In the FES condition, the input-output relation for the TA MEP was altered immediately after walking for 30 min and 10 min after walking (Fig. 5-2A). The MEP increased more at intermediate TMS intensities (50 and 60%), with little change at the maximum level. Fig. 5-2B shows input-output curves before, immediately after, and 10 min after walking from an experiment without FES in the same subject. The MEP did not significantly increase in amplitude at any levels of TMS, but tended to decrease at near the maximum level.

The MEP was normalized by the initial value (measured before walking) at each TMS intensity for each subject. Then, the normalized MEP_h and MEP_{max} were pooled to calculate group means of changes in the MEP. Fig. 5-3A shows group means ± SEs of the TA MEP_h. The MEP_h started increasing soon after initiation of walking with FES, and by the end of walking, reached 127±8% of the initial value (Student's t-test, $P<0.01$).

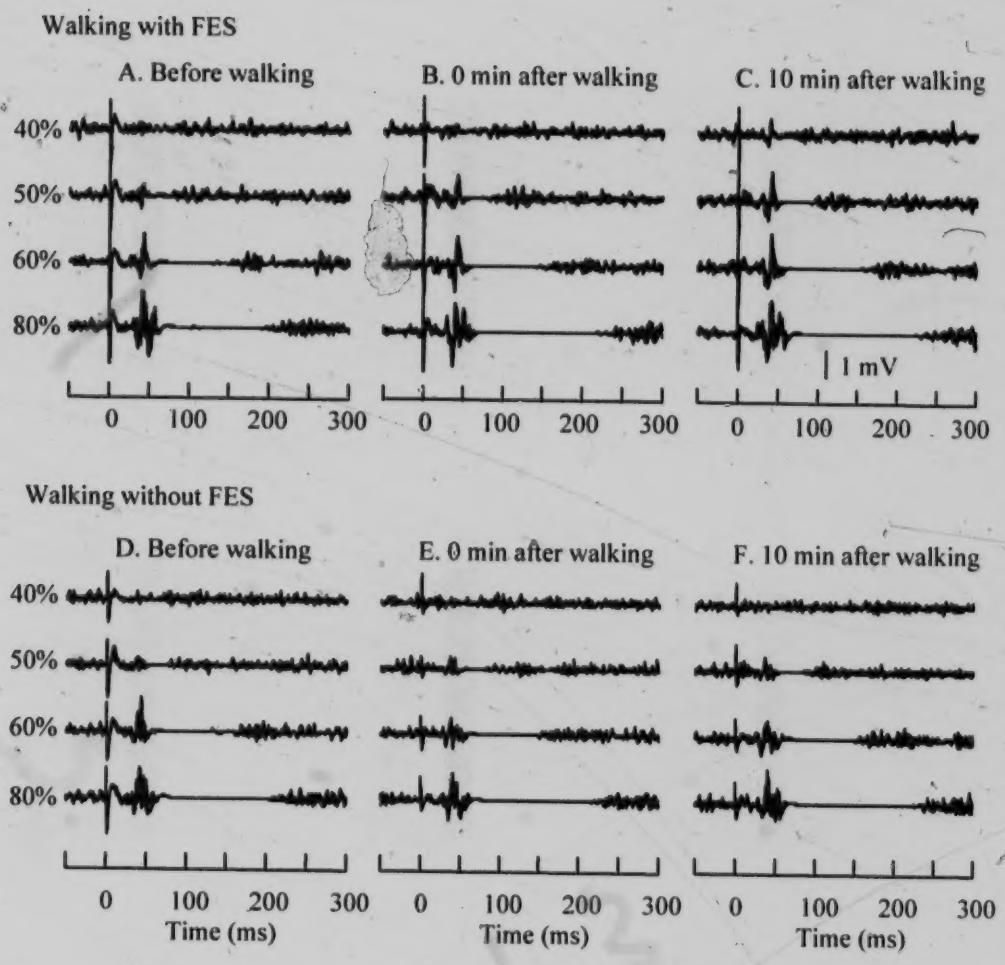

Fig. 5-1 Changes in the TA MEP with or without FES are shown in a single subject (MD). MEPs were measured during voluntary activation of the TA at 15% MVC. A-C show changes in the TA MEP before (A), immediately after (B), 10 min after (C) walking with FES for 30 min. The shading shows the position of the MEP. Following the MEP there is a silent period (SP) with little or no EMG activity. TMS intensities are indicated on the left of A and D. Four sweeps are superimposed in each panel. D-F show changes in the TA MEP before (D), 0 min after (E), 10 min after (F) walking.

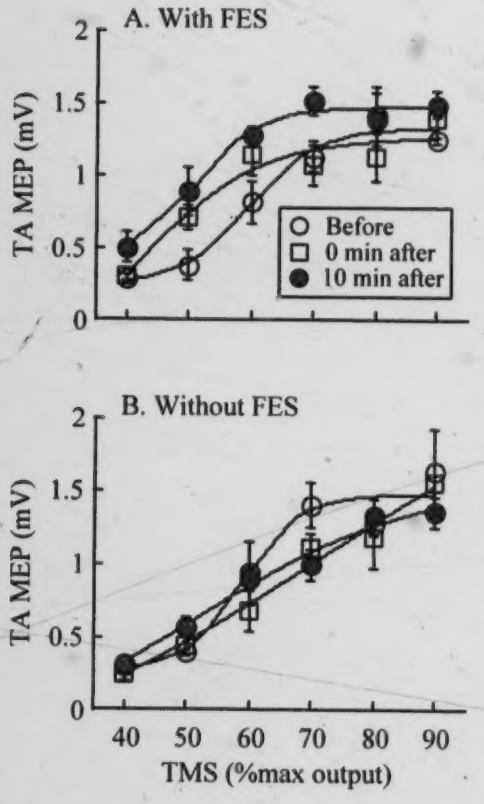

Fig. 5-2 MEP input-output curves in the TA muscle with FES (A) and without FES (B) for the subject in Fig. 5-1. Each point shows the average peak-to-peak MEP (mean ± SE of 4 responses). MEPs are shown before (○), immediately after 30 min of walking (□), and 10 min later (●).

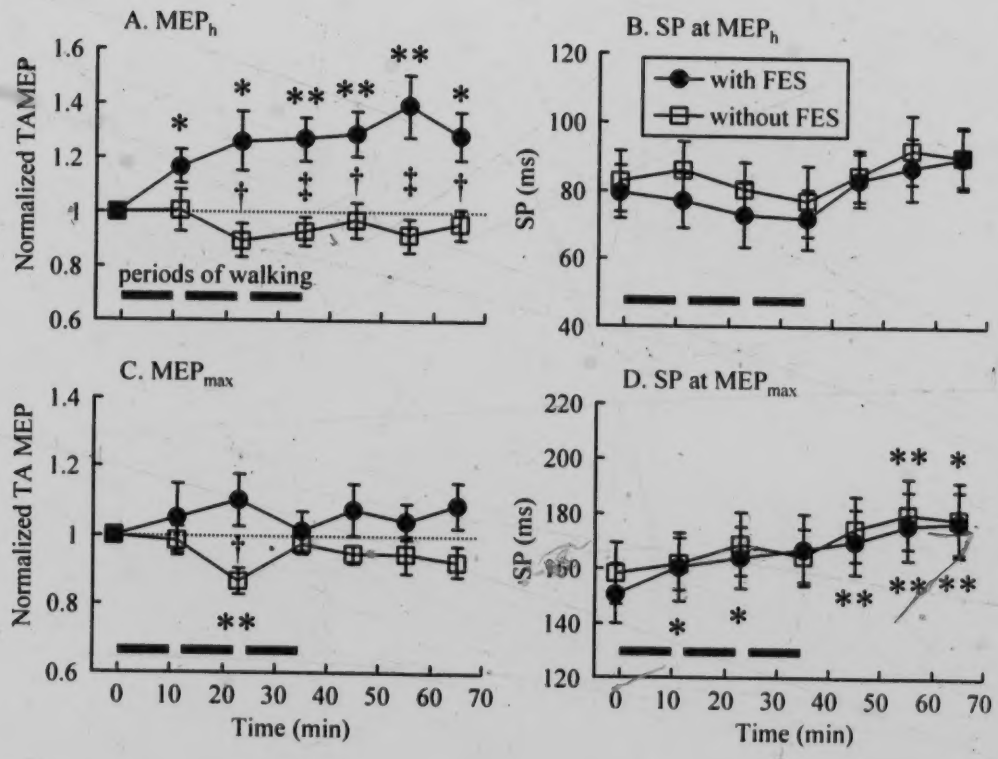

Fig. 5-3 TA MEPs and SPs are shown along the experimental elapsed time scale. Thick horizontal bars from 0 to 35 min show 3 × 10 min of walking with or without FES. MEPs measured during 15% MVC of voluntary contraction were normalized by the initial values for each subject. Circles show group means for walking with FES, and squares represent group means for walking without FES. Error bars indicate ± SE. A: Normalized means of MEP_h for walking with and without FES. B: Silent periods at MEP_h for the FES and non-FES conditions. C: Normalized means of MEP_{max} for walking with and without FES. D: Silent periods at MEP_{max} with and without FES. Significant differences from the baseline tested by Student's t test are indicated by * (P<0.05) or ** (P<0.01). Significant differences between the FES and non-FES conditions are indicated by † (P<0.05) or ‡ (P<0.01).

The MEP_h was largest 20 min after the end of walking (139±11%), although this value was not significantly different from that immediately after walking. In contrast, the MEP_h for the non-FES condition showed no significant change throughout the experiment. As a result, the values with and without FES became significantly different after 20 min of walking, and remained different at all later times. Group means of normalized MEP_{max} are shown for walking with and without FES in Fig. 5-3C. MEP_{max} for the FES condition did not change significantly at any time throughout the experiment. MEP_{max} for the non-FES condition did not increase, but decreased significantly in the middle of experiment. After 20 min of walking, the MEP_{max} was 87±4% of the initial value ($P<0.01$), and was significantly less than that with FES ($P<0.05$).

5.3.2 Silent period in the TA EMG

As can be seen in Fig. 5-1, the SP measured in the TA showed only a mild change after 30 min of walking with or without FES. Fig. 5-3B shows group means of SPs at MEP_h for walking with and without FES. Although results of one-way repeated measures ANOVAs indicated a significant effect of time on the duration of SP ($P<0.01$) with FES, the decrease or increase was not significant at any time (Student paired t test, $P>0.05$). Without FES, the effect of time was not significant (ANOVA, $P=0.07$). These small changes in the SP duration did not resemble changes in the MEP_h (see Fig. 5-3A). In fact, the ratio of normalized SP to MEP_h decreased over the experimental time course (Fig. 5-4). The SP-MEP_h ratio for the FES condition was significantly reduced after 10 min of walking (0.87±0.06, $P<0.05$), and became even less after more walking. After the end of walking with FES, the SP-MEP_h ratio gradually started to recover, and returned to the

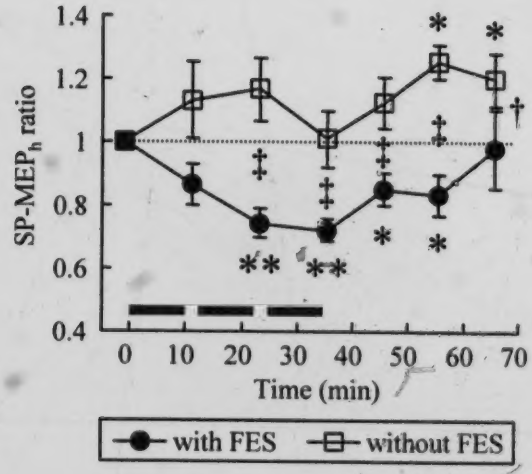

Fig. 5-4 The ratio of normalized SP to MEP_h did not increase in the FES condition. For symbols and bars, see Fig. 5-3. Significant differences from the baseline tested by Student's t test are indicated by * ($P<0.05$) ** ($P<0.01$). There were significant differences between conditions with and without FES after 20 min (†: $P<0.05$, ‡: $P<0.01$).

initial value at the end of experiment. In contrast, the SP-MEP_h ratio of the non-FES condition did not decrease but increased 20 min after walking (1.26±0.08, $P<0.01$) and remained high. Thus, there were significant differences between the FES and non-FES conditions after 20 min of walking until at least 30 min after walking ($P<0.05$).

SPs at MEP_{max} are shown for walking with and without FES in Fig. 5-3D. There were significant effects of time on the SP duration in both the FES and non-FES conditions (repeated measures ANOVA, $P<0.001$ for both). The SP for the FES condition started increasing 10 min after the initiation of walking, and kept increasing gradually until the end of experiments. The change in the SP for the non-FES condition was basically similar to that for the FES condition, and the increase was significant 20 and 30 min after walking ($P<0.05$). The maximum increase in the FES condition was 26.1±6.4 ms, 30 min after walking, and that in the non-FES condition was 21.6±6.6 ms, 20 min after walking. It was a little surprising that the SP for the MEP_{max} increased during the experiment, since the MEP_{max} did not increase over the time course of experiment. With the FES condition, the SP-MEP ratio for the MEP_{max} was significantly raised after 20 min of walking (1.15±0.04, $P<0.05$), and remained high after walking. The SP-MEP_{max} ratio also increased in the non-FES condition: 1.26±0.09 after 20 min of walking ($P<0.05$), and remained high after walking.

5.3.3 Changes in the soleus MEP and silent period

The soleus MEPs during weak tonic plantarflexions (15% MVC) were measured before and after 30 min of walking either with or without FES of the TA in 8 subjects. The background activity during the MEP measurement was constant throughout the

experiment (one-way repeated measures ANOVAs, $P>0.05$ at all TMS intensities). Group means ± SEs of normalized soleus MEP_h are shown in Fig. 5-5A. In contrast to a fairly consistent increase of the TA MEP_h, a change of the soleus MEP_h was quite variable among the subjects. Significant increases appeared 10 and 20 min after walking ($P<0.05$). In walking without FES, changes in the soleus MEP_h were not significant. Also, differences between the FES and non-FES conditions were not significant at any time. Fig. 5-5C shows normalized soleus MEP_{max} for walking with and without FES. The MEP_{max} showed some increase during the experiment, but the changes were not statistically significant.

The soleus SP showed a gradual increase after 30 min of walking with FES (Fig. 5-5B). Results of one-way repeated measures ANOVAs showed a significant effect of time on the duration of SP ($P<0.01$) for both conditions. With FES, SPs at MEP_h were significantly prolonged from 10 min after walking ($P<0.05$) to the end of experiment (31.4±10.5 ms) (Fig. 5-5B). Changes in the SP at MEP_h were smaller without FES, but significant 10 and 30 min after walking ($P<0.05$). Fig. 5-5D shows SPs at MEP_{max}. Although results of ANOVAs indicated significant effects of time on SPs ($P<0.05$ for both conditions), total increases in the SP were small: 15.5±8.2 ms for the FES condition (Student paired t test, $P=0.06$) and 15.2±4.0 ms for the non-FES condition ($P<0.01$). Note that P values might appear to be significant by chance since multiple comparisons were made between tasks. When ratios of SPs to MEPs were normalized by initial values, changes in the SP-MEP ratio were not significant at either MEP_h or MEP_{max} with or without FES.

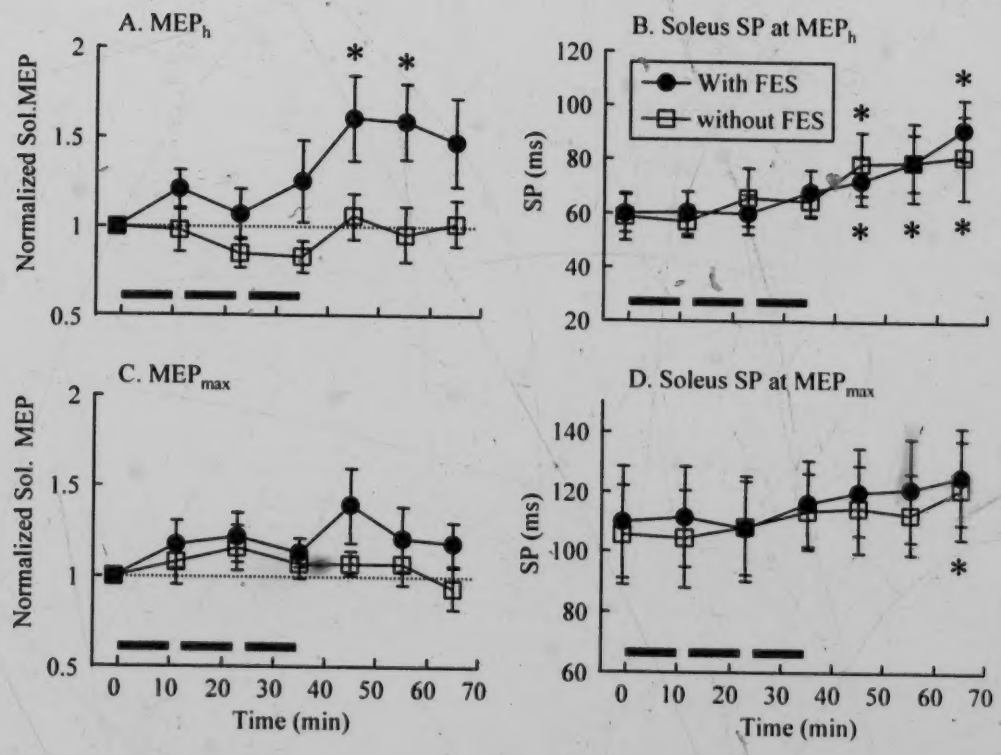

Fig. 5-5 A: Group means of normalized soleus MEP_h for walking with and without FES. B: Group means of soleus SPs at MEP_h with and without FES. C: Group means of normalized soleus MEP_{max} for walking with and without FES. D: Group means of soleus SPs at MEP_{max} with and without FES. For symbols and bars, see Fig. 5-3.

5.4 Discussion

The purpose of the present study was to investigate short-term effects of FES on the TA and soleus MEPs. After 30 min of walking with FES, the TA MEP_h (but not MEP_{max}) increased and this facilitatory effect lasted for at least 30 min. On the contrary, without FES, walking had no facilitatory effects on the TA MEP_h or MEP_{max}. With or without FES, the TA SP at MEP_h showed little change, although delayed increases were seen at MEP_{max}. The soleus MEP_h was also enhanced after walking with FES to the CP nerve, but not without FES. No significant difference was found in the soleus MEPs with and without FES.

5.4.1 Effect of walking on corticospinal excitability of the TA

One of the main questions addressed in this study was whether walking alone could enhance corticospinal excitability. As shown in Fig. 5-3A and C walking without FES did not increase the MEP_h or the MEP_{max} over the experimental time course, indicating that walking does not increase corticospinal excitability by itself. Schubert et al. (1998) reported a negative effect of walking on the TA MEP after 15 min of walking, the mean MEP area decreased by 34% in healthy subjects. In our study, the TA MEP_{max} was only significantly reduced at 20 min, so the 30 min of walking did not seem to produce substantial fatigue.

SPs were slightly prolonged 20 min after walking at both MEP_h and MEP_{max} (Fig. 5-3B and D), despite the fact that neither MEP_h nor MEP_{max} increased over the experimental period. As has been suggested in several studies, the SP and MEP probably originate from different cortical neurons (Ziemann et al. 1996; Ikeda et al. 2000;

Trompetto et al. 2001). Thus, on the assumption that SPs measured in the present study would more or less reflect cortical inhibition, walking may have some influence on cortical inhibition, at least, in the short term.

5.4.2 Effect of walking with FES on corticospinal excitability of the TA

The TA MEP_h started to increase after 10 min, and reached 128% of the initial value after 30 min of walking with FES (Fig. 5-3A). This facilitatory effect lasted for at least 30 min after FES ceased. What led to the increase of MEP_h? As described in *Results*, the background EMG level did not change. Change in the excitability of spinal motoneuron pools during the experiment is also unlikely, as other studies with similar, repetitive, nerve stimulation reported no changes in the H-reflex, short latency stretch reflex, F-wave, or MEP evoked by brainstem electrical stimulation (Ridding et al. 2000; Kaelin-Lang et al. 2002; Khaslavskaia et al. 2002; Knash et al. 2003). Therefore, the change of MEP_h is most likely due to the change in cortical excitability.

The MEP input-output curve has three main parameters: the maximum value, the stimulus level for the half-maximum, and the slope (Devanne et al. 1997). In the present study, the stimulus level for the half-maximum changed (as indicated by a change in the MEP_h amplitude) while the maximum value did not. This could be due to the left-shift of the whole curve, the change of slope, or the combination of both. We could not decide between these possibilities statistically, although after walking with FES MEPs were often present at a TMS intensity that was originally below the threshold (see Fig. 5-1A-C). Compared to the previous finding with the repetitive CP nerve stimulation (Knash et al. 2003), the rate of increase was slow. With repetitive stimulation of the CP nerve at rest,

the TA MEP_h significantly increased by 28% after 10 min of stimulation and by 43% after 30 min, whereas the increase was more gradual in the present study, 26% after 10 min and 27% after 30 min. Possibly, this is due to some negative effect of walking. Similar to the observation by Knash et al. (2003), the amplitude of MEP_{max} did not change over the time course of experiment. Thus, a short-term application of FES probably facilitates pre-existing connections, but does not create new synaptic connections, at least within the short time frame investigated in the present study.

While the SP at MEP_h did not show any significant changes, the SP at MEP_{max} increased without an increase of MEP_{max} both with and without FES. Since this change in the SP was similar with and without FES, this was mostly caused by walking and less likely related to FES. In fact, in a somewhat similar case, sustained submaximal muscle activation has been shown to influence the MEP and SP differently (Sacco et al. 1997).

5.4.3 FES versus repetitive peripheral nerve stimulation

Recently, Sinkjaer and Khaslavskaia (2002) reported that the effect of the repetitive CP nerve stimulation on the TA MEP could be enhanced by some voluntary activation of the target muscle. Is the FES walking any better than stimulation during sitting? The maximum increase in the TA MEP_h with FES (39%) was not different from that with the nonfunctional repetitive nerve stimulation (44%, Knash et al. 2003). In addition, the increase of MEP_h appeared more rapidly with the repetitive nerve stimulation than the actual FES. Thus, one might think that FES during walking is not as effective as the repetitive nerve stimulation in increasing corticospinal excitability. However, in the present study, the SP-MEP_h ratio decreased with FES (see Fig. 5-4). In

contrast, with stimulation during sitting Knash et al. (2003) found that the SP-MEP ratio increased. The SP has been shown to be due, at least partly, to cortical inhibition (Fuhr et al. 1991; Ziemann et al. 1993; Chen et al. 1999; Bertasi et al. 2000; Shimizu et al. 2000; Wu et al. 2000; Weber and Eisen 2002). Thus, unchanged or even slightly decreased SPs (immediately after 30 min of FES, -7.2 ±4.6 ms, P=0.10) accompanying increased MEP_h suggest unchanged or relatively suppressed cortical inhibition. As indicated in many studies, cortical inhibition is related to cortical plasticity, and reduced intracortical inhibition is important for inducing plastic changes in the cortex (Jacobs and Donoghue 1991; Ziemann et al. 1998a; Ziemann et al. 1998b; Ridding and Rothwell 1999; Butefisch et al. 2000; Ziemann et al. 2001; Chen et al. 2002; Kaelin-Lang et al. 2002; Levy et al. 2002). Finally, since cortical excitability increased with FES and inhibition did not, FES did not simply increase general excitability of the cortex, but likely had specific effects on different cortical neurons.

5.4.4 Changes in the soleus MEP

Not only the TA MEP, but also the soleus MEP showed significant increases with FES, although the difference with and without FES was not significant at any of the experimental time points. This is different from the results by Knash et al. (2003), in which the soleus MEP_{max} decreased and the MEP_h did not change (see also, Khaslavskaia et al. 2002). In the previous study, the soleus was not activated during or between the CP nerve stimulation, whereas in the present study, FES was applied in a phase-locked manner in relation to the soleus activation. At transition from the stance to swing phase

and the beginning of stance phase, there was some overlap between FES and soleus activation, which might increase the soleus MEP_h.

Overall, changes in the soleus SPs were quite small. SPs increased gradually as MEPs increased over the experimental period, resulting in no significant changes in the SP-MEP ratio at either MEP_h or MEP_{max}. In general, changes in corticospinal excitability seemed more or less specific to FES, whereas changes in cortical inhibition were nonspecific to FES, likely resulting from walking.

5.4.5 FES-aided locomotion and cortical plasticity

Only the combination of locomotor activity and FES led to the increase of corticospinal excitability without increased cortical inhibition, which may enhance reorganization of the motor cortex or strengthening some of the synaptic connections within a certain cortical area. Many cortical areas and corticospinal descending pathway are activated during locomotion (Nathan 1994; Jiang and Drew 1996; Schubert et al. 1997; Kably and Drew 1998; Keck et al. 1998; Schubert et al. 1999; Petersen et al. 2001; Drew et al. 2002; Malouin et al. 2003). Concurrently, cutaneous and muscle afferent activity is fed back in the CNS. Thus, integration of descending and afferent inputs in the CNS may facilitate cortical plasticity. Likewise, in the present study the TA activation level during FES-aided walking was much higher than that during regular walking. Increased activation of the TA will increase afferent inputs to the CNS, and thereby, might influence plasticity. During locomotion more muscle groups are recruited and EMG amplitudes are higher than during attempted voluntary movement in spinal cord injured patients (Maegele et al. 2002). Therefore, a combination of locomotor activity

and functional electrical stimulation might effectively facilitate plastic changes in the CNS of patients after spinal cord injury, stroke, or other CNS disorders, but this remains to be tested in future experiments.

5.5 References

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6. SHORT-TERM EFFECTS OF FUNCTIONAL ELECTRICAL STIMULATION ON SPINAL EXCITATORY AND INHIBITORY REFLEXES IN LEG MUSCLES

6.1 Introduction

Functional electrical stimulation (FES) of residual neural pathways below the lesion has been used to restore lost function in patients with central nervous system disorders. The long-term use of lower limb FES has been shown to increase muscle strength, fatigue resistance, bone density, and muscle oxidative capacity (Stein et al. 1992; Greve et al. 1993; Rochester et al. 1995; Gerrits et al. 2000; Stein et al. 2002) and reduce spasticity (Levin and Hui-Chan 1992; Granat et al. 1993; Solomonow et al. 1997). A short-term application of therapeutic electrical stimulation (TES) has also been shown to attenuate spasticity (Walker 1982; Seib et al. 1994; Potisk et al. 1995; Dewald et al. 1996). In addition, regularly applied FES of the common peroneal (CP) nerve substantially improves walking speed in hemiplegic patients (Stein et al. 1993; Taylor et al. 1999; Wieler et al. 1999; Barbeau et al. 2002), likely due, at least partly, to alteration in neural connections at the spinal and/or supraspinal level over the period of FES use. Overall, FES and FES-assisted muscle use seem to induce plastic changes at both the muscle and neural levels.

Previously, we have shown short-term effects of FES-aided walking on corticospinal excitability in healthy subjects (Kido Thompson and Stein 2004). FES was applied to the CP nerve during the swing phase of the step cycle when the ankle flexors are active, and motor evoked potentials (MEPs) by transcranial magnetic stimulation were measured in the tibialis anterior (TA) muscle before, between and after periods of

walking with FES. After 30 min of walking with FES, the half-maximum peak-to-peak MEP in the TA increased and this facilitatory effect lasted for at least 30 min. The silent period, which is known to reflect at least partly cortical inhibition (Fuhr et al. 1991; Chen et al. 1999; Werhahn et al. 1999), did not increase while the TA MEP increased, suggesting that FES does not simply increase general excitability of the cortex, but had specific effects on particular cortical neurons. However, the origin of increased corticospinal excitability could not be specified, as the spinal excitability was not tested. Similarly, repetitive stimulation of the peripheral nerve has been shown to induce the motor cortex reorganization and an increase of corticospinal excitability (Hamdy et al. 1998; Ridding et al. 2000; Ridding et al. 2001; Kaelin-Lang et al. 2002), without increasing spinal reflex excitability (Khaslavskaia et al. 2002; Knash et al. 2003). These studies suggest that the increased corticospinal excitability is cortical in origin.

While the effect of peripheral nerve stimulation on corticospinal pathways has been investigated extensively, the effect on spinal, especially inhibitory connections has drawn little attention. As noted above, after the long-term use of FES or short-term application of TES, spasticity was reduced in spastic patients, suggesting effects of peripheral nerve stimulation on spinal circuits. However, to date, it is not known whether the combination of electrical stimulation and functional movement (i.e., FES) has any short-term effects on excitatory and/or inhibitory spinal reflex excitability.

The purpose of the present study was to examine short-term effects of FES-assisted walking on inhibitory and excitatory spinal reflexes elicited in the ankle flexors and extensors. In this study, the electrical CP nerve stimulation was given during the swing phase of the step cycle (i.e., at functionally relevant times), and therefore, is

referred to as functional electrical stimulation (FES). FES was delivered using the WalkAide2 foot drop stimulator (Stein 1998). Several different spinal reflexes in the TA and soleus muscles were measured before, between, and after periods of walking with FES: the TA and soleus H-reflexes, reciprocal inhibition measured as depression of ongoing EMG activity, and reciprocal and presynaptic inhibition of the soleus H-reflex by conditioning CP nerve stimulation.

Ashby and Wiens (1989) studied reciprocal inhibition of ongoing TA EMG activity by the tibial nerve stimulation in spinal cord injured patients, and found that the TA inhibition was greater and had a lower threshold in patients than in normal subjects. Thus, we wished to examine whether reciprocal inhibition would change after a short-term application of FES in healthy subjects. The H-reflex has been used to test spinal α-motoneuronal excitability and excitability of different spinal pathways interacting with the H-reflex pathway (Capaday and Stein 1987; Schieppati 1987; Misiaszek 2003). An exaggerated stretch reflex (and its electrical analogue H-reflex) has been a well-known common feature of spasticity (Delwaide 1987; Brown 1994; Hiersemenzel et al. 2000), and if FES has any effects on spasticity in the short-term, it might be observed as a reduction of the H-reflex amplitude, together with the changes in other spinal reflexes. In spastic patients, impaired reciprocal inhibition of the soleus has also been reported (Crone et al. 1994; Boorman et al. 1996; Okuma et al. 2002; Crone et al. 2003), and recently, Perez et al. (2003) showed a transient effect of patterned CP nerve stimulation on Ia reciprocal inhibition of the soleus H-reflex. Thus, in the present study we tested whether FES, another patterned stimulation of the CP nerve during functional movement, could result in a similar increase of Ia reciprocal inhibition. In addition, reduced or abnormal

presynaptic inhibition has been proposed as one of the mechanisms involved in spasticity (Yang et al. 1991; Nielsen et al. 1995; Morita et al. 2001). Therefore, we studied whether FES has any effects on presynaptic inhibition of the soleus H-reflex.

6.2 Materials and Methods

6.2.1 General procedure

Fourteen neurologically normal subjects aged from 20 to 63 participated in this study. All subjects gave informed consent for the purposes and procedures of the experiments, as approved by the Human Ethics Committee of the University of Alberta.

Electromyograms (EMG) were recorded from the TA and soleus muscles. Initially the maximum tonic voluntary contraction (MVC) of each muscle was determined as the maximum rectified EMG level during standing. Resistance was manually applied so that the ankle angle did not change during the MVC measurement. The subject was asked to voluntarily maintain a target muscle EMG level to match a preset level on an oscilloscope during nerve stimulation (see *EMG recordings and electrical stimulation*).

First, in order to obtain the whole soleus H-M recruitment curve, the tibial nerve was stimulated at various stimulus intensities during standing with a tonic EMG activity of 15% MVC for the soleus muscle. Subsequently, 8-16 responses were averaged at the stimulus intensity for the maximum soleus H-reflex (H_{max}). Then, inhibition of the ongoing EMG activity was elicited during 15-75% MVC of tonic TA contraction. In the present study, 1.5 × motor threshold (MT) of the tibial nerve stimulation was used to test inhibition, since this intensity of stimulation produces clear inhibition in most healthy subjects, and is sensitive to task- and age-dependent modulation (Kido et al. 2004b; Kido

et al. 2004a). The same protocols were used for the CP nerve stimulation and the inhibition of soleus voluntary activity.

The soleus H-reflex was conditioned by stimulating the CP nerve at 1.5 × MT prior to the test tibial nerve stimulation. The short test stimulus delay (<4 ms) was for testing reciprocal inhibition (Crone et al. 1987; Crone et al. 1990; Perez et al. 2003), and the long delay (90-120 ms) for presynaptic inhibition (Capaday et al. 1995; Zehr and Stein 1999; Earles et al. 2001). A test tibial nerve stimulus intensity eliciting ≅50% H_{max}, corresponding to 29.4±8.2% M_{max} (mean ± SD, n=10), was used for the H-reflex conditioning paradigm, since this size of H-reflex is sensitive to both inhibitory and facilitatory modulation (Zehr and Stein 1999; Kido et al. 2004a) see also (Crone et al. 1990). Eight to 10 responses were averaged for both conditioned and unconditioned (i.e., control) H-reflexes during standing with ≅15% MVC of the soleus EMG activity.

In the present study, we tested 6 different spinal reflexes in a standing position: inhibition of the ongoing EMG activity in antagonist muscle of the tibial and CP nerves, the soleus and TA H_{max}, and short- and long-latency inhibition of the soleus H-reflex. While the soleus H_{max} was tested in all 14 subjects, 2 or 3 different measurements were made in each subject, since it was difficult to perform all 6 kinds of testing at a time. After the first set of measurements, the subject walked on a treadmill at 4.5 km/h for 15 min with FES of the CP nerve, eliciting approximately the half maximum M-wave in the TA. Then, the second set of reflex measurements was made. After another 15 min (i.e., a total 30 min) of walking with FES, 3 more sets were measured: immediately, 15 min, and 30 min after FES-assisted walking. The stimulus to evoke the H-reflex or inhibition was

carefully maintained at each measurement to produce the same relative intensity over an experimental period.

On separate days, effects of walking (alone) on spinal reflexes were examined in 7 subjects, using the same protocol but no FES. Also, in 6 subjects we tested effects of repetitive CP nerve stimulation, which is similar to FES but with no movement associated. Details of experimental setup for repetitive nerve stimulation have been described elsewhere (Knash et al. 2003). Basically, the CP nerve was repetitively stimulated at 25 Hz with 50% duty cycle (800ms on and off) while the subjects was at rest, seated in a chair with the foot strapped in a custom-made apparatus. The stimulus intensity was maintained to produce the half maximum-M-wave in the TA EMG.

6.2.2 EMG recordings and electrical stimulation

The soleus and TA EMG signals were obtained using surface self-adhesive Ag-AgCl electrodes (Vermont Medical, Inc., Bellows Falls, VT). EMG recording electrodes were placed 2-3 cm below the gastrocnemius in line with the Achilles tendon for the soleus, and over the muscle belly for the TA, with ~2 cm interelectrode spacing. The signals were amplified, high-pass filtered at 10 Hz, low-pass filtered at 1 kHz, and recorded with Axoscope (Axon Instrument, Union City, CA) at a sample rate of 5 kHz. EMG signals were also rectified, low-pass filtered at 3 Hz, and sent to an oscilloscope, so that subjects could monitor their EMG activity levels during experiments.

The electrical stimulation was applied to the CP nerve and/or the tibial nerve by surface electrodes (Vermont Medical, Inc., Bellows Falls, VT). The stimulus electrode for the CP nerve for reflex testing was placed at a low threshold point near the lateral

edge of popliteal fossa, and the anode electrode was located ~2 cm anterior to the cathode. For the tibial nerve stimulation the cathode was placed in the popliteal fossa and the anode ~2 cm higher. The test stimulation was a 1 ms rectangular pulse delivered from Grass SD9 isolated stimulators (Grass Inst., Quincy MA), which was triggered by a pseudo-random pulse generator with intervals of 1.6 to 2.7 s.

6.2.3 Functional electrical stimulation

In the present study, the subject was asked to walk on a treadmill with FES. WalkAide2 (Stein 1998) is a single channel FES device that has been developed to prevent foot drop by stimulating ankle flexors during the swing phase of walking for patients with various CNS disorders. The WalkAide 2 is triggered by a tilt sensor (Stein 1998), and stimulates the CP nerve near the head of the fibula at 25 Hz. Stimulation begins when the leg is tilted backwards at the end of stance and ends when the leg is tilted forward at the end of swing. The stimulus intensity was adjusted to produce approximately the half maximum M-wave in the TA EMG, which was similar to what we used previously (Kido Thompson and Stein 2004). We did not observe any obvious changes in the walking pattern between the stimulated and unstimulated legs and no subjects reported fear or danger of falling during FES-assisted walking.

6.2.4 Data analysis

Details of evaluating inhibition of the ongoing EMG activity by the antagonist muscle nerve stimulation have been described elsewhere (Kido et al. 2004b; Kido et al. 2004a). Basically, the amount of inhibition was expressed as the difference between the

mean rectified EMG for a 7-10 ms period including the peak inhibition and the background EMG calculated for a 50 ms prestimulus period. We refer to this as reciprocal inhibition without specifying neural pathways that may be involved, since the inhibition may contain disynaptic and other short latency reflex components. The amount of inhibition was then normalized by the background EMG. Since there is a linear relation between the background EMG and the amount of inhibition, (Capaday et al. 1990; Kido et al. 2004b), normalized inhibition from different background levels was pooled together to calculate the mean value of normalized inhibition for each subject. Sixteen to 24 responses were averaged in each subject.

The peak-to-peak soleus and TA H_{max} amplitudes were measured before, between and after periods of walking. To evaluate the relative amount of change across different subjects, H_{max} amplitudes were also normalized by the initial H_{max} values.

Short- and long-latency inhibition of the soleus H-reflex can be seen as a decrease of the H-reflex after conditioning CP nerve stimulation (Mizuno et al. 1971; Tanaka 1974; Crone et al. 1987; Crone et al. 1990). The size of conditioned H-reflex was expressed as % control H-reflex, and changes in reciprocal (i.e., short-latency) or presynaptic (long-latency) inhibition could be observed as a change in the conditioned H-reflex. That is, the stronger the inhibition, the smaller the conditioned H-reflex.

For statistical analysis, a Student's paired t-test was used to assess the difference between periods before and after walking with FES, walking alone, or FES-like stimulation. Since we were interested only in the change from the baseline measurement, we did not test changes between any other times. Normalized H_{max} was evaluated by

Student's one-sample t-test; significance of a change in the H_{max} was determined by whether the value was different from 1.

6.3 Results

In the following, effects of FES-assisted walking are mainly presented, since it was our main interest. Effects of repetitive CP nerve stimulation or walking without FES are summarized in Table 6-1, and discussed later in Results section.

6.3.1 Inhibition of ongoing EMG activity

Inhibition of the ongoing soleus EMG activity by the CP nerve stimulation was measured in 13 subjects during standing. The group means ± SEs of normalized inhibition are shown in Fig. 6-1. Basically, there was no significant change in the soleus inhibition over the experimental period (Student's paired t-test, $P>0.05$). Similarly, inhibition of the TA EMG by the tibial nerve stimulation did not show any significant increase or decrease after walking with FES (n=10, $P>0.05$). Thus, walking with FES for 30 min did not have any significant effects on reciprocal inhibition of ongoing EMG activity tested by the antagonist muscle nerve stimulation.

6.3.2 Soleus and TA H-reflexes

The soleus H_{max} was examined in all 14 subjects who participated in this study. Fig. 6-2A and B show a typical change in the soleus H_{max} after walking with FES in a single subject. The change in amplitude was small, but 13 of 14 subjects showed a similar decrease; the initial H_{max} was 4.44±0.70 mV (mean ± SE), and after 30 min of

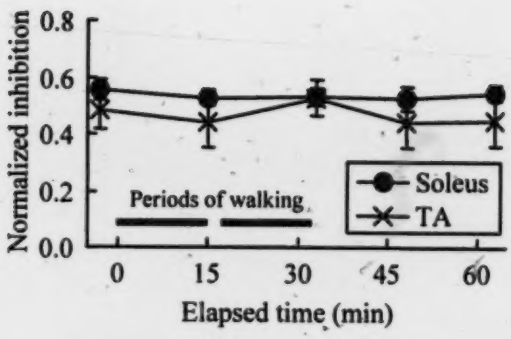

Fig. 6-1 Inhibition of the ongoing EMG activity by the antagonist muscle nerve stimulation. Filled circles show group means of normalized soleus inhibition (n=13) before, between, and after periods of walking with FES. Crosses represent group means of normalized TA inhibition (n=10). Error bars indicate ± SE. Thick horizontal bars indicate periods of walking.

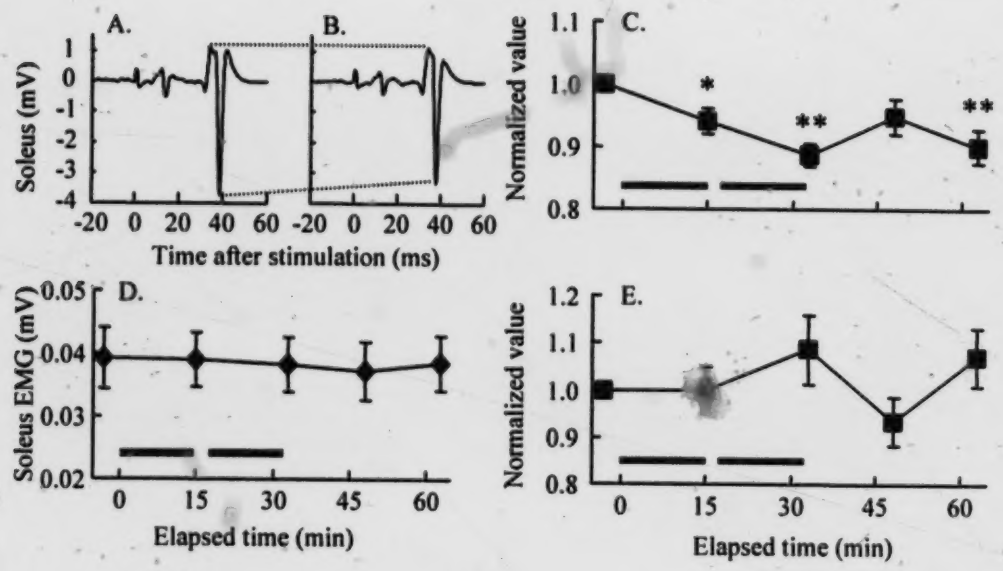

Fig. 6-2 The soleus H_{max} decreased slightly but significantly after walking with FES. The change in the TA H_{max} was small and did not show any trends. A and B: the soleus H_{max} before (A) and after (B) walking with FES for 30 min in subject E.D. C: the group means ± SE of normalized soleus H_{max} (n=14) before, between, and after periods of walking with FES. D: the group means ± SE of the soleus background EMG during the H_{max} measurements. E: the group means ± SE of normalized TA H_{max} (n=11). Significant differences from the baseline tested by Student's t test are indicated by * (P<0.05) and ** (P<0.01).

walking with FES the H_{max} became 3.96±0.61 mV (Student's paired t test, $P<0.01$). The group mean ± SE of normalized H_{max} was 0.89±0.02 after walking, indicating ≅11% reduction (Fig. 6-2C), and this small reduction in the soleus H-reflex lasted at least 30 min after FES-assisted walking ($P<0.01$). Since the soleus background EMG level was carefully maintained and did not change over the experimental period (Fig. 6-2D, Student's paired t test between before and after walking with FES, $P>0.1$), the decrease of the H_{max} was not due to a change in the motoneuronal background excitability. The H_{max}/M_{max} ratio was also decreased after 30 min of walking with FES in 4 subjects tested (from 0.65±0.07 to 0.57±0.07, Student's paired t test, $P<0.05$). Thus, it is unlikely that the decrease of H_{max} was associated with a fatigue at the neuromuscular junction.

The TA H_{max} was also measured in 11 subjects. Compared to the soleus H_{max}, it was quite small (the initial amplitude: 1.06±0.19 mV), and there were no consistent changes across different subjects over the experimental period (Fig. 6-2E).

6.3.3 Short- and long-latency inhibition of soleus H-reflex

The short-latency reciprocal inhibition of the H-reflex was tested during standing in 8 subjects. As shown in Fig. 6-3A, the size of conditioned H-reflex became smaller after 30 min of FES-assisted walking. The conditioned H-reflex, expressed as a % control of the H-reflex decreased statistically from 77.8±3.4% to 71.2±5.4% (mean ± SE) after walking. However, this increase of reciprocal inhibition was no longer significant 15 min after, similar to the finding by Perez et al. (2003).

The long-latency presynaptic inhibition of the H-reflex was examined in 10 subjects. As can be seen in Fig. 6-3B, the conditioned H-reflex did not change

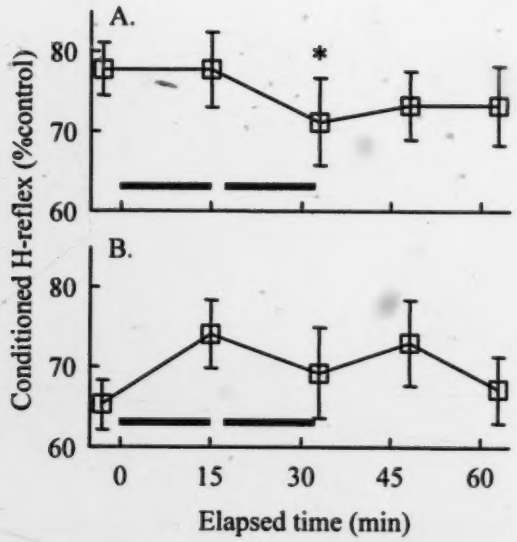

Fig. 6-3 The short-latency reciprocal inhibition of the soleus H-reflex slightly changed after walking with FES. A: the group means ± SE of soleus H-reflexes conditioned by the CP nerve stimulation with short delays (n=8) before, between, and after periods of walking with FES. Note that a decrease of conditioned H-reflex implies an increase in inhibition. B: the group means ± SE of conditioned H-reflexes with long stimulus delays to test presynaptic inhibition (n=10). Thick horizontal bars indicate periods of walking. For a symbol, see Fig. 6-2.

significantly from the initial measurement over the experimental period. Thus, 30 min of FES-assisted walking had no significant effects on presynaptic inhibition.

6.3.4 Separate effects of repetitive CP nerve stimulation at rest and walking

Six different spinal reflex responses measured before, between, and after periods of FES-like repetitive CP nerve stimulation or walking are summarized in Table 6-1 (from top, inhibition of the ongoing soleus EMG activity, inhibition of the TA EMG activity, the normalized soleus H_{max}, the soleus background EMG during the H_{max}, the normalized TA H_{max}, postsynaptic reciprocal inhibition of the soleus H-reflex, and presynaptic inhibition of the soleus H-reflex). Similar to the results of FES-assisted walking, the TA H-reflex did not change after repetitive CP stimulation (Knash et al. 2003) or walking. Inhibition of the ongoing EMG activity did not show any significant changes in either the soleus or TA, confirming the finding from walking with FES (see Fig. 6-1). Interestingly, the soleus H_{max}, again, showed a small but significant decrease after 30 min of walking. Stimulation alone seemed to decrease the soleus H-reflex, but not to a significant extent. Different from the observation with FES-assisted walking (Fig. 6-3), reciprocal inhibition of the soleus H-reflex did not change significantly after either repetitive CP stimulation or walking alone. This was, however, not too surprising, as a small statistical increase in reciprocal inhibition of the H-reflex after FES was transient and not seen as inhibition of the ongoing EMG activity (Fig. 6-1). Presynaptic inhibition of the soleus H-reflex tended to decrease (i.e., increase in size of the conditioned H-reflex) after walking, but did not reach a statistical significance.

Table 1 Effects of repetitive CP nerve stimulation or walking on spinal reflexes

		before	15 min	30 min	45 min	60 min
Soleus	rCP	0.67 ± 0.04	0.65 ± 0.03	0.63 ± 0.02	0.60 ± 0.04	0.62 ± 0.02
inhibition	walk	0.55 ± 0.03	0.60 ± 0.04	0.58 ± 0.03	0.58 ± 0.03	0.63 ± 0.03
TA	rCP	0.50 ± 0.07	0.46 ± 0.07	0.48 ± 0.06	0.51 ± 0.07	0.43 ± 0.09
inhibition	walk	0.57 ± 0.06	0.61 ± 0.06	0.64 ± 0.07	0.63 ± 0.08	0.60 ± 0.09
Soleus	rCP	1	1.01 ± 0.03	0.94 ± 0.05	0.89 ± 0.04	0.90 ± 0.04
H-reflex	walk	1	0.90 ± 0.06	0.83 ± 0.05 *	0.89 ± 0.04 *	0.89 ± 0.06
Soleus	rCP	0.033 ± 0.006	0.036 ± 0.006	0.032 ± 0.006	0.035 ± 0.006	0.039 ± 0.011
BG (mV)	walk	0.036 ± 0.006	0.034 ± 0.006	0.036 ± 0.005	0.037 ± 0.005	0.038 ± 0.005
TA	rCP	1	1.10 ± 0.13	0.97 ± 0.10	1.08 ± 0.10	1.07 ± 0.21
H-reflex	walk	1	1.08 ± 0.14	0.96 ± 0.11	1.07 ± 0.11	1.11 ± 0.14
Cond. H	rCP	68.0 ± 10.2	70.7 ± 11.6	85.2 ± 5.4	79.1 ± 10.1	81.8 ± 6.9
postsyn.	walk	68.7 ± 7.7	76.3 ± 6.6	78.1 ± 5.0	74.6 ± 7.7	76.8 ± 7.0
Cond. H	rCP	71.6 ± 7.6	71.2 ± 9.0	71.2 ± 8.8	72.9 ± 7.6	82.7 ± 7.8
presyn.	walk	55.6 ± 9.1	79.7 ± 5.1	74.2 ± 7.5	72.5 ± 8.7	67.4 ± 7.2

Different spinal reflex responses were measured before, after 15 min of stimulation or walking (15 min), after 30 min of stimulation or walking (30 min), 15 min after the end of stimulation or walking (45 min), and 30 min after (60 min). All the values are shown as mean ± SE, except for baseline values for the normalized soleus and TA H-reflexes. Cond. H stands for conditioned H-reflex and values are shown as %control reflex amplitude. postsyn. = postsynaptic reciprocal inhibition, presyn. = presynaptic inhibition, rCP = repetitive CP nerve stimulation. *: significantly different from the baseline value (paired t-test, $P<0.05$).

6.4 Discussion

In the present study short-term effects of FES-assisted walking on inhibitory and excitatory spinal reflexes were investigated. Inhibition of ongoing EMG activity by the antagonist muscle nerve stimulation, the TA H-reflex, and presynaptic inhibition of the soleus H-reflex did not show any significant changes after walking with FES of the CP nerve. The soleus H-reflex decreased slightly but significantly after FES-assisted walking. Reciprocal inhibition of the soleus H-reflex showed a small transient increase after 30 min of FES-assisted walking, which, however, could not be reproduced by either stimulation alone or walking alone. Overall, the observed changes were quite small (i.e., less than 15% of increase or decrease), compared to previous findings in corticospinal excitability after FES (Kido Thompson and Stein 2004).

6.4.1 Effect of FES on spinal inhibition

The short-latency reciprocal inhibition of the soleus H-reflex transiently increased after 30 min of FES-assisted walking, similar to the finding by Perez et al. (2003). However, the change in the conditioned H-reflex amplitude was small: from 78% to 71% control H-reflex, and could not be reproduced by either stimulation alone (at rest) or walking without stimulation (Table 6-1). Furthermore, this small increase of reciprocal inhibition could not be detected as inhibition of the ongoing soleus EMG activity, another (and possibly more functional) measure of reciprocal inhibition (Lavoie et al. 1997). As mentioned above, the inhibition measured as a depression of ongoing EMG activity in the present study may contain disynaptic and other short-latency reflex components, and therefore, a small change that happens within a few milliseconds period may not be

reflected in a measure calculated for a ~10 ms period. Indeed, the fact that we did not find a significant increase in a gross measure of reciprocal inhibition of the soleus suggests that FES-assisted walking would not have a strong impact on reciprocal soleus inhibition. Similarly, the TA inhibition by the tibial nerve stimulation did not change over the experimental period. Presynaptic inhibition of the soleus H-reflex did not show consistent changes after FES across different subjects. Presynaptic inhibition has been studied in several different ways (Faist et al. 1994; Capaday et al. 1995; Meunier and Pierrot-Deseilligny 1998; Zehr and Stein 1999; Perez et al. 2003), and other presynaptic inhibitory pathways might be more sensitive to FES. However, Perez et al. (2003) reported no change in D1 inhibition after 30 min of patterned CP nerve stimulation (10 pulses at 100Hz every 1.5 s, below or at the threshold). In addition, in the present study, none of the inhibitory pathways examined showed significant changes after repetitive CP stimulation at rest or walking with no stimulation. Thus, the currently available observations suggest that 30 min of FES-assisted walking would not have strong effects on spinal inhibitory pathways, which are important for movement coordination.

6.4.2 Effect of FES on the H-reflex

In the present study, the soleus H_{max} significantly decreased after walking with FES. Although it was small (≅11%), a similar extent of decrease was seen in most of the subjects tested. Since a similar decrease of the H_{max} was seen after walking, the reduced H_{max} might be due to walking itself, although the CP nerve stimulation at rest also seemed to decrease the H-reflex to a minor extent. Whatever the cause was (i.e., FES-assisted walking, stimulation, or walking), a decrease of the soleus H_{max} after FES-

assisted walking cannot be explained by a small transient increase of reciprocal inhibition after FES, since there was no relation in the extent of decrease between the soleus H_{max} and inhibition of the H-reflex in 8 subjects in whom reciprocal inhibition was tested. Presynaptic inhibition of the H-reflex also showed no significant change over the experimental period. In addition, the soleus H_{max} remained decreased for at least 30 min after FES (Fig. 6-2C), which was quite different from what could be seen in pre-and post synaptic inhibition of the H-reflex and soleus inhibition (compare Fig. 6-2 with 6-1 and 6-3, and see also Perez et al. 2003). The origin of this modification of the soleus H_{max} is unknown.

As mentioned above, an exaggerated stretch or H-reflex is one of the common features of spasticity (Delwaide 1987; Brown 1994; Hiersemenzel et al. 2000) and improved spasticity might be observed as a reduction of the reflex size (Walker 1982; Levin and Hui-Chan 1992). However, recently the causal relation between reflex hyperexcitability and spasticity has been questioned (Dietz 1997; Dietz 2002; O'Dwyer and Ada 1996; O'Dwayer et al. 1996; Sinkjaer et al. 1993). Thus, whether a short-term use of FES leads to a temporary reduction of extensor spasticity in a patient population cannot be concluded from the present observation of a decrease in the soleus H-reflex with no significant changes in examined inhibitory reflexes.

The TA H_{max} did not change significantly after 30 min of FES-assisted walking, walking alone, or stimulation alone. This is similar to the findings by Khaslavskaia et al. (2002) and Knash et al. (2003) after 30 min of repetitive patterned stimulation, and suggesting that 30 min of FES of the CP nerve would not increase the reflex excitability of the TA motoneuron at the spinal level.

6.4.3 Effects of FES on spinal and supraspinal connections

In the previous study, we showed short-term effects of walking with FES on corticospinal excitability in healthy subjects (Kido Thompson and Stein 2004). After 30 min of FES-assisted walking, the TA MEP increased significantly and this facilitatory effect lasted for at least 30 min after FES. An increase of the TA MEP is due to increased corticospinal excitability, which could be spinal motoneuronal, cortical, or a combination of both. The present result showed that spinal reflex excitability for the TA did not change after FES, thereby supporting the hypothesis that a single session of FES-assisted walking increases cortical excitability for the TA. A possible mechanism of a selective facilitatory effect of FES on corticospinal connectivity, rather than spinal excitability, has been proposed by Rushton (2003). He postulated that if synapses between corticospinal neurons and motoneurons are Hebb-type synapses, coincidence of voluntary effort through pyramidal tract neurons and activation of motoneurons via orthdromic volleys from FES of peripheral nerve axons may modify/strengthen specific neural connections. This might be one of the mechanisms involved in functional recovery after long-term use of FES (Stein et al. 1993; Taylor et al. 1999; Wieler et al. 1999; Barbeau et al. 2002). However, in a short term, we have not found a clear difference between FES given during locomotion and nonfunctional electrical stimulation delivered at rest, in terms of strengthening corticospinal connectivity (Knash et al. 2003; Kido Thompson and Stein 2004).

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7. SPINAL RECIPROCAL INHIBITION BETWEEN ANKLE FLEXOR AND EXTENSOR MUSCLES AFTER CHRONIC CNS LESIONS

7.1 Introduction

Lesions in the CNS often lead to locomotor disorders (Dietz 1997; Dietz 2002) and one of the most common problems in hemiplegic patients is foot drop (Knutsson 1981; Burridge et al. 2001). Foot drop may arise from reduced corticospinal descending inputs. Corticospinal connections seem to be stronger to the ankle flexors than to the extensors (Brouwer and Ashby 1992; Bawa et al. 2002), and corticospinal control of the tibialis anterior (TA) muscle is active during walking (Schubert et al. 1997; Capaday et al. 1999; Petersen et al. 2001). Thus, damage to the CNS may affect activation of the flexor muscles more than that of the extensors, and is evident as foot drop. However, in addition to very little flexor activity during the swing phase (especially near the toe-off or heel contact), calf muscle activities are also often abnormal during walking in patients with foot drop (Knutsson 1981; Dietz et al. 1994; Burridge et al. 2001). Reduced calf muscle EMG activity at the push-off (i.e., at the end of the stance phase) is quite common in the hemiplegic (Burridge et al. 2001), as well as stretch reflex responses at the beginning of the stance phase, in response to passive dorsiflexion (Yang et al. 1991; Burridge et al. 2001). Yang et al. (1991) also reported no or little modulation of the soleus H-reflex during walking in those subjects; that is, the H-reflex was present in the swing phase. Probably this is due to abnormal presynaptic inhibition (see also, Capaday and Stein 1989; Stein et al. 1993; Yang and Whelan 1993), and together with improper activation of the flexor muscles, may contribute to foot drop. However, modification of

presynaptic inhibition might depend on the pathology and the pathway tested (Faist et al. 1994; Nielsen et al. 1995; Katz 1999; Aymard et al. 2000; Morita et al. 2001).

In addition to exaggerated excitatory responses after CNS lesions (i.e., spasticity; (Taylor et al. 1984; Delwaide 1987; Thilmann et al. 1991; Brown 1994) different spinal inhibitory pathways operating at the ankle joint might be altered in hemiplegic patients. For instance, Ib inhibition of the soleus by the medial gastrocnemius nerve stimulation was replaced by facilitation (Delwaide and Oliver 1988). Recurrent inhibition of the soleus was increased in spinal cord injured (SCI) patients (Shefner et al. 1992) and in hemiplegia due to stroke (Katz and Pierrot-Deseilligny 1982).

Reciprocal inhibition between the ankle extensors and flexors has been studied in different patient populations with different methodologies. Ashby and Wiens (1989) studied Ia inhibition of the TA by stimulating the posterior tibial nerve, and found greater inhibition with lower threshold in incomplete SCI than in normal subjects. Reciprocal inhibition of the soleus H-reflex by the common peroneal (CP) nerve stimulation was also increased in the spastic incomplete SCI (Boorman et al. 1991). However, when the soleus H-reflex was conditioned by voluntary contraction of the pretibial muscles, this "natural reciprocal inhibition" was less in the SCI (Boorman et al. 1996). Reciprocal Ia inhibition of the soleus was absent in multiple sclerosis (MS) patients (Crone et al. 1994), and replaced by facilitation in hemiplegia due to stroke or SCI (Crone et al. 2003). Different observations may arise from differences in the severity of damage and the extent of recovery (Okuma and Lee 1996; Okuma et al. 2002). Thus, it seems that spinal reciprocal inhibition is likely altered after a CNS lesion. However each study examined only one direction of inhibition (i.e., either inhibition of the TA by the tibial nerve or

inhibition of the soleus by the CP nerve) and the two directions of reciprocal inhibition between the tibial and CP nerves have not been tested on the same individuals. Also, most observations were made within 5 years after the onset of CNS damage. Although, from a clinical perspective, a patient's condition often reaches a stable state 6-12 months after a CNS lesion, some patients report gradual functional recovery over a few years. Spinal pathways might be modified during the limited functional recovery over several years. So far, to our knowledge, spinal reciprocal inhibition between ankle extensors and flexors has not been examined thoroughly in long-standing chronic hemiplegic subjects.

The purpose of the present study was to investigate spinal reciprocal inhibition of ankle extensor or flexor muscles in chronic hemiplegic subjects with foot drop. The inhibition was measured as a depression of the ongoing EMG activity produced by the antagonist muscle nerve stimulation. Although conditioning the soleus H-reflex by the CP nerve has been a well established method to test spinal inhibition (Mizuno et al. 1971; Tanaka 1974; Crone et al. 1985; Crone and Nielsen 1989; Crone et al. 1990), with the current method, the amount of inhibition could be evaluated regardless of the presence or size of the H-reflex. This allowed us to study reciprocal inhibition at all ages noninvasively (Kido et al. 2004a), even in the ankle flexor muscles. The amount of reciprocal inhibition measured in the hemiplegic patient was also compared to that in age-matched control subjects, which has been published elsewhere (Kido et al. 2004a).

7.2 Materials and methods

7.2.1 Subjects

Fifteen adult subjects with chronic CNS lesions (age 31-72) who had foot drop

participated in this study. All subjects gave informed consent for the purposes and procedures of the experiments, as approved by the Human Ethics Committee of the University of Alberta. For this study, subjects with different pathologies were included as long as all of the following criteria were satisfied: (1) adult onset; (2) medically stable for more than 6 months since the last episode of stroke or injury; (3) adequate stability at the ankle joint during standing; (4) adequate cognitive and communication function to give informed consent; (5) able to ambulate with or without an assistive device; (6) inadequate dorsiflexion during the swing phase of gait, resulting in toe drag or inadequate limb clearance; (7) no lower motoneuronal damage at the lumbosacral level.

7.2.2 General procedure

Electromyograms (EMG) were recorded from the tibialis anterior (TA) and soleus muscles with surface self-adhesive Ag-AgCl electrodes (Vermont Medical, Inc., Bellows Falls, VT). At the beginning of each experiment, the maximum tonic voluntary contraction (MVC) of each muscle was determined as the maximum rectified EMG level during standing. Then, the subject was asked to voluntarily control a target muscle EMG level for either plantar flexion or dorsiflexion to match a preset level on an oscilloscope (see the section *EMG recordings and electrical stimulation*). Reciprocal inhibition of the ankle extensors and flexors was elicited by stimulating the common peroneal (CP) or the tibial nerve respectively during standing.

First, the effect of CP nerve stimulus intensity on inhibition of the soleus EMG activity was investigated. Under fixed tonic contraction (about 15% MVC) of the soleus, the CP nerve stimulus intensity was increased from below the TA motor threshold (MT)

to the maximum M-wave. Four responses were recorded at each stimulus level, and collected data were immediately analyzed to determine the MT of the CP nerve stimulation, using a custom-written program in MATLAB (Mathworks Inc., Natick, MA). Then, inhibition was produced at several different soleus tonic contraction levels using 1.5 × MT of the CP nerve stimulation, which previously produced clear inhibition in healthy subjects (Kido et al. 2004b). While contraction levels were voluntarily changed, M-wave amplitudes were maintained to keep a steady level of stimulation. The same protocols were used for the tibial nerve stimulation and the resulting inhibition of the TA voluntary activity.

7.2.3 EMG recordings and electrical stimulation

The soleus and TA EMG signals were obtained using surface Ag-AgCl electrodes (2.2 × 3.5 cm, Vermont Medical, Inc., Bellows Falls, VT). EMG recording electrodes were placed 2-3 cm below the gastrocnemius in line with the Achilles tendon for the soleus, and over the motor point for the TA, with ~2 cm interelectrode spacing. The signals were amplified, high-pass filtered at 10 Hz, low-pass filtered at 1 kHz, and recorded with Axoscope (Axon Instrument, Union City, CA) at a sample rate of 5 kHz. EMG signals were also rectified, low-pass filtered at 3 Hz, and sent to an oscilloscope, so that subjects could monitor their EMG activity levels during experiments. EMG and nerve stimulus signals were recorded for 300 ms, including a prestimulus period of 50 ms, in response to each test stimulus pulse.

The CP or the tibial nerve was stimulated via surface Ag-AgCl electrodes (2.2 × 2.2 cm, Vermont Medical, Inc., Bellows Falls, VT) to inhibit the antagonist muscle activity.

The stimulus electrode for the CP nerve was placed at a low threshold point near the neck of the fibula where activation of the TA was prominent and activation of peroneal muscles was weak, and the anode electrode was located below the patella. The electrodes for the tibial nerve stimulation were placed in the popliteal fossa (cathode) and 2 cm higher (anode). Stimuli were 1 ms rectangular pulses delivered from Grass SD9 isolated stimulators (Grass Inst., Quincy MA). The test stimulation was triggered by a random pulse generator, so that the stimuli were generated with pseudo-random intervals (1.6 to 2.7 s).

7.2.4 Data analysis

The effect of antagonist muscle nerve stimulation on the target muscle was measured as the difference between the background and a selected period for inhibition in the ongoing EMG activity. The target muscle EMG was rectified, and then, the background level was calculated for a 50 ms prestimulus period. Inhibition was measured as the mean level of EMG amplitude for a 7-10 ms period including the peak depression (Fig. 7-1). When the period of inhibition appeared to be longer than 20 ms, the first 10 ms was used for analyses. Usually depression of the EMG activity appeared within15 ms after onset of the H-reflex in the stimulated muscle. When it was difficult to find depression in the EMG, a period for analysis was determined in relation to the onset latency for the H-reflex. For example, the subject of Fig.7-1 had the TA H-reflex onset latency of 37 ms (not shown in figure), and the following soleus inhibition appeared 48-58 ms after the CP nerve stimulation. In this subject K.R., the tibial nerve stimulation did not produce inhibition in the TA EMG at any stimulus intensities. Therefore, we

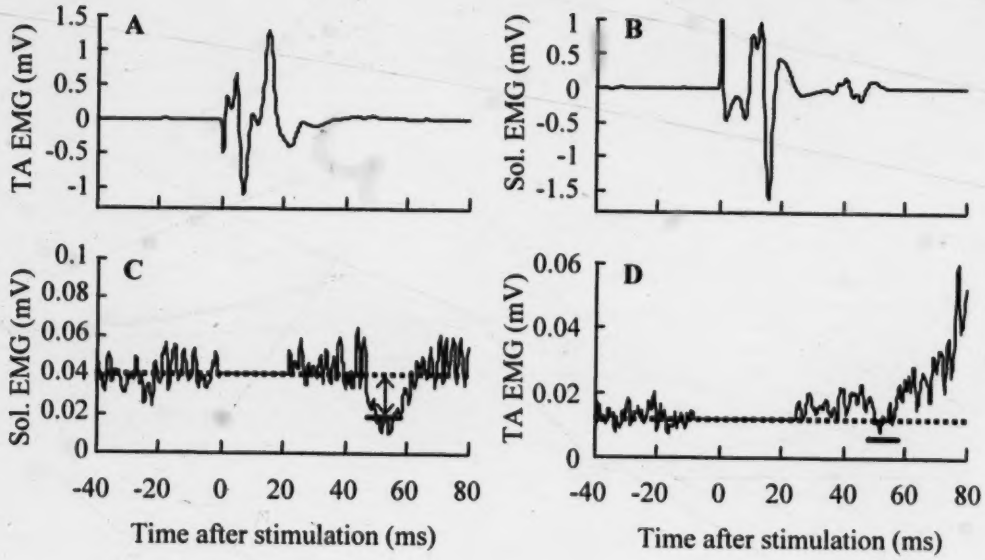

Fig. 7-1 Reciprocal inhibition of the ongoing EMG activity following the antagonist muscle nerve stimulation (1.5 × MT) in a subject with MS (K.R.). A and C: the TA EMG and the rectified soleus EMG recorded in response to the CP nerve stimulation. The mean level of inhibition (mV) was measured for a 7-10 ms window (see short bar) including the peak depression in the rectified EMG. (The amount of inhibition is indicated as an arrow.) The stimulus artifact and contamination from the M-waves of nearby muscles have been removed for clarity. B and D: the soleus EMG and the rectified TA EMG activity after the tibial nerve stimulation. Note that there is little or no inhibition at the expected time (see horizontal bar). A longer latency excitation was sometimes seen, but has not been analyzed here. The dotted horizontal line indicates the background EMG level. Twenty sweeps were averaged for each part of the figure.

measured his soleus H-reflex latency, which was 37 ms, and with the same delay for inhibition from the H-reflex as that for the soleus inhibition, a period for the TA inhibition was determined; 48-58 ms after stimulation was used for analyzing the TA response in K.R.. From our previous experience of measuring reciprocal inhibition in healthy subjects, it seemed reasonable to assume that the delay of inhibition from the H-reflex in the antagonist muscle would be similar in both the soleus and TA. The inhibition was measured over a period of time and may contain disynaptic and other short latency reflex components. Therefore, we refer to this as reciprocal inhibition, without specifying neural pathways that may be involved.

The amount of inhibition was also normalized by the background EMG. As shown in previous studies (Capaday et al. 1990; Petersen et al. 1999; Kido et al. 2004b), there is a positive linear relation between the background EMG level and the amount of inhibition. Thus, normalized inhibition from different background EMG levels could be pooled together to calculate the mean value of normalized inhibition for each subject. In general, the subject aimed to generate >4 different levels of EMG activities within the range of 10-85% MVC as indicated on the oscilloscope, and the number of responses averaged was 16-24 in each subject. Normalized inhibition in chronic subjects was, then, plotted with the mean value for control subjects (age 40-70). Data in healthy subjects have been published elsewhere (Kido et al. 2004a), and only age-matched controls were used here for comparison.

7.3 Results

Table 7-1 presents the profiles of the subjects. Some subjects were affected in both legs, but we studied the more affected leg. As expected, the stroke subjects were older (59.3±4.5; mean ± SE) than the other subjects (44.0±2.7). Also, because of the reduced life expectancy of the older stroke subjects, the time after onset of symptoms was generally less (4.3±1.3, compared to 16.2±4.2 years). One of the characteristics of spasticity, an abnormally large H-reflex, compared to age-matched controls, was often seen in these subjects; the higher maximum H/maximum M (H_{max}/M_{max}) ratios were found in 8 of 11 subjects tested. However, there was no statistical difference in the size of the H-reflex or the inhibitory reflexes between the stroke and other subjects. Therefore, for the rest of the results data for all subjects will be combined.

7.3.1 Voluntary activation of the soleus and TA muscles

Rectified EMG amplitudes for MVC measured during standing are shown for the soleus and TA muscles in Fig. 7-2. Most of the subjects studied had smaller soleus MVC amplitudes (Fig. 7-2A) compared to age-matched control subjects (thick horizontal line). Reduced voluntary EMG activity was even more marked with the TA MVC (Fig. 7-2B), likely contributing to the foot drop. Since surface electrodes were used, the smaller amplitudes found in some subjects might partly be due to skin resistance or electrode placement. However, experimental protocols were essentially the same for both affected and control subjects. Thus, these small EMG amplitudes indicate weakened voluntary activation of both the soleus and TA muscles.

Table 7-1 Patients' characteristics

	age (yr)	sex	pathology	after onset (yr)	H_{max} (mV)	H_{max}/M_{max}
M.M.	71	M	ischaemic stroke	0.6	0.36	0.14
L.M.	54	F	hemorrhagic stroke	1	0.31	0.26
S.J.	40	F	hemorrhagic stroke	1.3	NA	NA
C.B.	31	F	SCI (C5-6)	1.5	5.39	1.00
E.J.	40	M	SCI (C6-7)	2	NA	NA
G.T.	72	M	ischaemic stroke	4	NA	NA
K.B.	69	M	ischaemic stroke	6	1.87	0.76
D.Z.	57	F	hemorrhagic stroke	8	11.43	NA
B.B.	52	M	hemorrhagic stroke	9	3.81	0.41
C.B.	37	F	MS	9	5.77	0.80
K.R.	52	M	MS	13	1.77	0.45
M.L.	43	M	head injury	19	2.37	0.60
J.H.	47	F	SCI (T12-L2)	24	6.27	0.90
B.W.	52	M	SCI (C5-6)	28.5	0.49	0.36
R.N.	50	M	SCI (C4-5)	32.5	8.21	0.85

* H_{max} (mV) and H_{max}/M_{max} are measured in the soleus during standing (i.e., generating ≅15% MVC of soleus EMG activity). NA means not available.

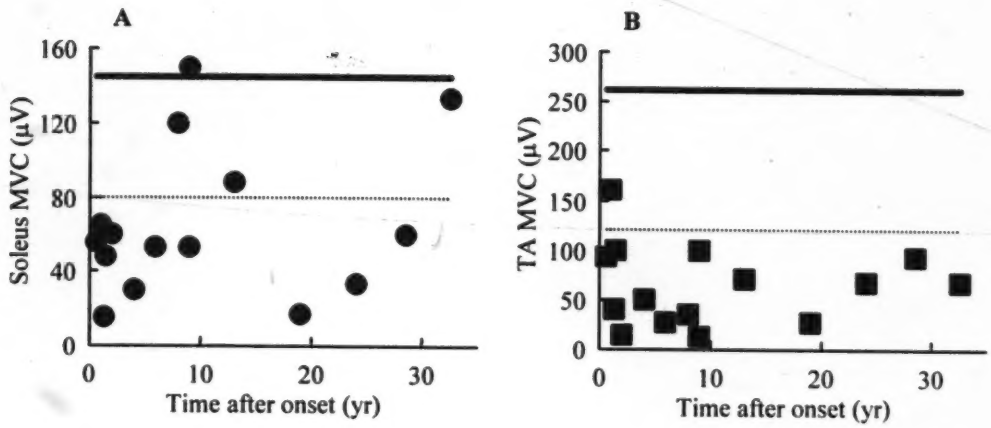

Fig. 7-2 Rectified EMG amplitudes for the soleus (A) and TA (B) MVC measured during standing are presented in relation to the time after onset of condition. Thick horizontal lines indicate the mean MVCs for healthy subjects (age 40-70), and dotted lines indicate lowest values in the control group, which were found in the oldest subject (age 70). No significant trends were found between MVC amplitudes and years after onset.

7.3.2 Soleus inhibition

Inhibition of the soleus ongoing EMG activity was examined in all 15 subjects with CNS lesions. When the amount of inhibition was measured with a fixed soleus contraction (≅15% MVC), the amount of inhibition in 9 subjects was positively correlated to the TA M-wave amplitude evoked by a variety of CP nerve stimulus intensities. That is, the stronger the stimulation the more inhibition in the majority of subjects examined. In the rest of subjects, who did not show significant trends between the stimulus intensity and inhibition, inhibition was often present when the stimulation was set at 1.5 × MT.

Normalized inhibition (see *Methods*) was plotted as a function of years after onset of condition in Fig. 7-3A. The soleus inhibition could be elicited by 1.5 × MT of the CP nerve stimulation in most of the hemiplegic subjects. Interestingly, the two subjects who showed the least inhibition were at a relatively early chronic stage (i.e., ≤1 year after onset), and many of the subjects who had been disabled more than 10 years showed very clear inhibition of the soleus. This is unlikely due to the stimulus intensity of 1.5 × MT used in this study. Fig. 7-4A and B show the soleus inhibition by the CP nerve stimulation in two different individuals. The CP nerve stimulation just below the threshold could not produce any inhibition in Subject L.M. who had stroke 1 year before (Fig. 7-4A). In this subject, any stimulus intensities tested (i.e., from 0.9–1.8 × MT) did not produce noticeable inhibition. In contrast, the CP nerve stimulation which was similarly weak but just above the threshold intensity produced clear inhibition in subject R.N. who had spinal cord injury 30 years before (Fig. 7-4B). The stimulus intensity-inhibition relation in R.N. was, in fact, not different from that in healthy controls (Fig. 7-4E) (Kido et al. 2004a). As a whole, inhibition in long-standing subjects (>10 years after

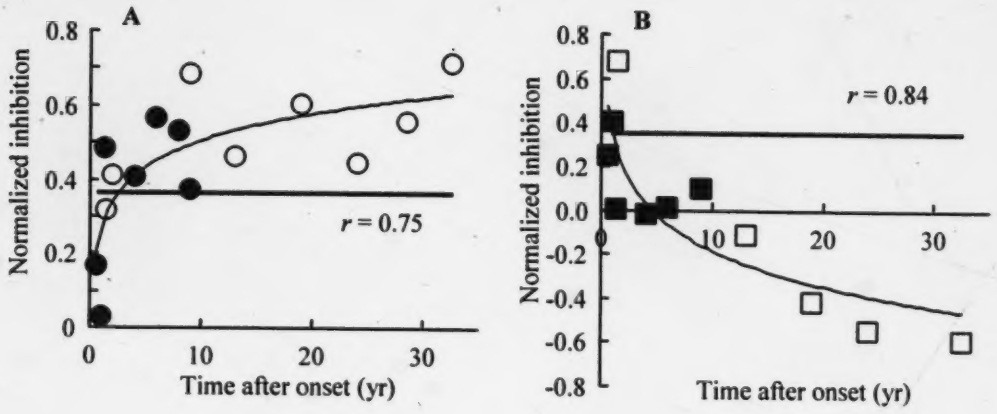

Fig. 7-3 Reciprocal inhibition of the ongoing EMG activity by 1.5 × MT of the antagonist muscle nerve stimulation in all subjects is shown as a function of the time after onset of condition. Thick horizontal straight lines indicate the mean values of normalized inhibition for healthy subjects (age 40-70). Filled symbols are for individuals who had stroke, and open symbols for individuals with other pathologies, such as SCI. A logarithmic curve was fitted to the group data and the Pearson's r value is presented for each condition. A: Inhibition of the soleus EMG activity by the CP nerve stimulation. The amount of inhibition, calculated as the difference between the background (BG) EMG and the mean rectified EMG of a selected 7-10 ms window, is normalized by the BG and averaged across different BG levels for each subject. B: Inhibition of the TA EMG activity by the tibial nerve stimulation. Negative values indicate excitation, instead of inhibition.

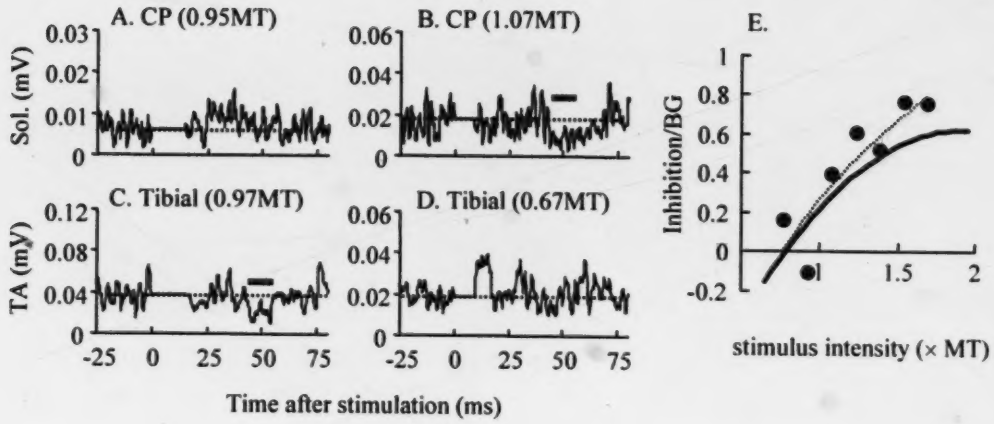

Fig. 7-4 Reciprocal inhibition of the ongoing EMG during 15% MVC of voluntary activation by the antagonist muscle nerve stimulation is presented in single subjects. A and B: The effect of weak CP nerve stimulation on the soleus EMG. Four sweeps were averaged. Stimulus intensities were 0.95 × MT for A, and 1.07 × MT for B. A period for inhibition is indicated by a thick horizontal grey bar. C: The effect of weak tibial nerve stimulation on the TA EMG. Stimulus intensities were 0.97 × MT for C, and 0.67 × MT for D. A and C are from subject L.M. and B and D are from subject R.N.. E: Inhibition of the soleus EMG activity by the CP nerve stimulation is shown as a function stimulus intensity in subject R.N.. Note that the relative amount of inhibition (i.e., normalized by the background EMG) is used for the ordinate. Each point represents the mean of 4 responses. A dotted line is fitted for subject R.N.'s data using the least squares method ($r = 0.88$). A thick line shows a trend in healthy subjects (derived from Kido et al. 2004).

onset) tended to be stronger than in control subject at similar ages (see Fig. 7-3A). The positive trend between the soleus inhibition and the time after onset was significant (*Pearson's r*=0.75, $P< 0.01$).

7.3.3 TA inhibition

Inhibition of the TA ongoing EMG activity could be examined in 11 out of 15 subjects. We could not test this inhibition in four subjects, because they could not activate the TA voluntarily during standing (see also Fig. 7-2B). On the attempt to produce the TA inhibition during ≅15% MVC of the TA activation, we found a significant trend between the amount of inhibition and the stimulus intensity in only one patient. This was not surprising, since the positive relation between the TA inhibition and the tibial stimulus intensity is not as clear as that for the soleus inhibition even in healthy subjects (Tomaru et al. 2003; Kido et al. 2004b). However, in control subjects inhibition was present when the stimulation was increased to 1.5 × MT (Kido et al. 2004b). In contrast, many of the subjects studied in the present study showed no signs of inhibition at any intensity.

The normalized TA response was expressed as a function of years after onset of CNS damage in Fig. 7-3B. Opposite to the observation in the soleus inhibition, 1.5 × MT of the tibial nerve stimulation could not elicit inhibition of the ongoing TA EMG, and it was replaced by strong excitation in some subjects. Most interestingly, the TA inhibition was present at a relatively early chronic stage (i.e., ≤1.5 year after onset, see Fig. 7-4C), and then, became progressively reduced over 10 years. Moreover, inhibition in long-standing chronic subjects (>10 years after onset) was replaced by excitation. With

weaker stimulus intensities, we had similar observations. Fig. 7-4C and D show the TA inhibition in two individuals at different chronic stages. The subthreshold level of the tibial nerve stimulation elicited inhibition in the TA EMG of subject L.M. who was at 1 year after onset (Fig. 7-4C). Whereas in subject R.N. (30 years after injury), a similar stimulation produced not inhibition but slight excitation (not shown in figure), and a further weaker stimulation did not produce any excitation but no inhibition (Fig. 7-4D). Although the TA inhibition in control subjects also decreased with age (Kido et al. 2004a), there was no directional change from inhibition to excitation with healthy aging. The negative trend between the TA inhibition and the time after onset was highly significant (*Pearson's* r=0.84, P<0.01).

7.4 Discussion

In the present study, spinal reciprocal inhibition of ankle extensor or flexor muscles measured as depression of the ongoing EMG activity produced by the antagonist muscle nerve stimulation was investigated in subjects with chronic foot drop. Inhibition of the soleus EMG was relatively small in subjects ≤1 year after onset, but was strong in long-standing subjects (>10 years after incident). In contrast, inhibition of the TA EMG was present at a relatively early, chronic stage (i.e., ≤1.5 year after onset), but gradually decreased over ≅10 years, and was eventually replaced by strong excitation. These findings suggest that abnormalities in reciprocal inhibition caused by CNS lesions might be changed gradually over several years to a few decades, and alteration in reciprocal inhibition might not be directly related to foot drop.

7.4.1 Inhibition of the soleus by the CP nerve stimulation

Strong inhibition of soleus activity by stimulating the CP nerve in long-standing chronic subjects was unexpected. Since all of the subjects studied had obvious foot drop, we anticipated that the reflexes might be decreased. Previous studies have used conditioning of the H-reflex as a measure of inhibition from the CP nerve and reported an increase (Boorman et al. 1991), a decrease or even replacement of the inhibition by facilitation (Crone et al. 1994; Crone et al. 2003). The use of the H-reflex conditioning in this patient group has problems, because the size of the H-reflex is often changed after CNS damage (Taylor et al. 1984; Okuma and Lee 1996) and the response is known to be quite sensitive to the level of the H-reflex (Crone et al. 1985; Crone et al. 1990). In addition, the H-reflex amplitude decreases with age (Sabbahi and Sedgwick 1982; Brooke et al. 1989; Koceja et al. 1995; Kido et al. 2004a) and the H-reflex is difficult to elicit in some older subjects (see also Table 7-1). Another difference from previous studies is that many of our subjects were examined more than 7 years after onset, whereas in some other studies subjects were examined soon after the onset of CNS damage (Boorman et al. 1996; Crone et al. 2003). We showed that there is a gradual, but marked change in the inhibitory reflexes with time. However, Crone et al. (1994) examined MS patients whose disease duration was 2-31 years after onset, and reported no inhibition in those patients. We have examined only two MS subjects, and the recurring nature of the disease makes it difficult to measure the time course of changes that evolve after a specific lesion. Another possible difference is the severity of the condition. Patient selection can influence the results (Okuma and Lee 1996; Okuma et al. 2002). All of our

subjects were ambulatory, in spite of a disturbing foot drop. Thus, they may have been functionally more homogeneous, although the etiology of the condition was diverse.

Is there a functional advantage for maintaining this inhibitory pathway in chronic hemiplegia? Certainly, controlling inhibition of the soleus (i.e., ankle extensors) is useful during voluntary activation of the TA (i.e., ankle flexors) (Shindo et al. 1984; Crone and Nielsen 1989; Nielsen and Kagamihara 1992). However, this inhibitory pathway will not be activated effectively in 'natural' motor control situations in patients with foot drop. Thus, having this soleus inhibition available (or not lost) may be good, but most of those patients will not make good use of it, without a stimulator for activation.

7.4.2 Inhibition of the TA by the tibial nerve stimulation

In the present study, inhibition of the TA existed in an early stage after chronic CNS lesions (i.e., ≤1.5 year after onset). This is not contradictory to findings by Ashby and Wiens (1989). Those authors found greater inhibition of the TA for a given stimulation with lower threshold in the incomplete SCI (7 months to 3 years after injury) than in normal subjects. Their evidence of increased Ia reciprocal inhibition from the tibial nerve to the TA offered a mechanism for weakened ankle dorsiflexion in CNS disorders. To our surprise, in the present study, the TA inhibition decreased over 10 years after onset and was eventually replaced by excitation. This was the most unexpected finding, as all of the subjects studied had foot drop. We expected that reciprocal inhibition of the TA might be increased in our chronic subjects, as found by Ashby and Wiens (1989).

There were some possible methodological reasons for not being able to detect the TA inhibition. First, in the present study, the EMG recording was made using surface electrodes. Ashby and Wiens (1989) detected EMG activity of single motor units with needle electrodes and measured peristimulus histograms and averaged over a population. Surface EMGs allow a population of units to be studied at once (Capaday et al. 1990; Petersen et al. 1999; Kido et al. 2004b; Kido et al. 2004a), and it is unlikely that an extreme difference such as inhibition and excitation is simply due to different EMG recording techniques. Secondly, the stimulus intensity used in this study (i.e., 1.5 × MT) may be so strong that the stimulus current could spread and activate the CP nerve near the popliteal fossa, resulting in an H-reflex in the TA. To minimize this possibility, we chose the tibial nerve stimulus location carefully so as not to activate the TA. Also, the TA H-reflex latency was shorter than the latency for the TA inhibition, so it is hard to believe that the measured inhibition/excitation was an evoked H-reflex. Moreover, as in the subject of Fig. 7-4D, in long-standing chronic subjects (>10 years after onset) who had excitation, instead of inhibition, in response to the tibial nerve stimulation at 1.5 × MT, we could not elicit inhibition at any lower stimulus intensities. Therefore, it is unlikely that the TA excitation reported here was an accidentally elicited H-reflex in the TA. Thirdly, we could not test the TA inhibition in some of the subjects, since their voluntary TA control was too weak to generate a maintained TA EMG activity. TA inhibition might be present in some of them but could not be measured. Fourthly, reduced inhibition might be partly explained by aging effects (Earles et al. 2001; Kido et al. 2004a) and reduced amount of physical exercise (Nielsen et al. 1993) in the patients. However, none of the above-mentioned factors alone or in combination can account for

the TA excitation found in long-standing chronic subjects, which was not found in age-matched controls.

Inhibition of the TA by the tibial nerve was present within the first few years after the onset, and then, replaced by excitation over the next decade. The reason for this is unknown. From a functional aspect, activating this inhibitory pathway is not needed in patients with foot drop, since the TA was very weak and often barely activated during walking. Excitation might be produced when the soleus muscle is stretched during the swing phase. Whether this excitation is important in compensating in part for the weakness in the TA remains to be studied.

7.4.3 Potential effect of stimulating the CP nerve

In the present study, the soleus inhibitory pathway could be activated in most of the chronic subjects. However, it is less likely that the CP nerve-soleus inhibitory connection is effective in their daily activities, as their voluntary activation of the ankle flexors is very limited. Instead, generating inhibitory inputs to the ankle extensors by means of the CP nerve stimulation might be considered to reduce unwanted activation of the extensors (Burridge and McLellan 2000), such as during the swing phase and swing-stance transition of the step cycle in locomotion. Also, hyperexcitability of the spinal stretch reflex arc is one of the common features of spasticity, and often seen in the lower limb extensor muscles. Although spasticity might be a result of increased non-reflex mediated stiffness and reflex-mediated stiffness might not be affected in some of the spastic patients (Sinkjaer et al. 1993; O'Dwyer and Ada 1996; Dietz 2000), there are individuals with spastic movement disorder, such as foot drop, showing a sign of reflex

hyperexcitability (see Table 7-1). Thus, those spastic individuals with reflex hyperexcitability may receive some benefit from repeatedly activating this inhibitory pathway. Indeed, application of functional or therapeutic electrical stimulation has been reported to reduce spasticity (Walker 1982; Levin and Hui-Chan 1992; Seib et al. 1994; Dewald et al. 1996; Solomonow et al. 1997). As mentioned in the beginning, abnormal calf muscle activity, such as reduced activity at the end of the stance phase (i.e., push-off) and stretch reflex responses at the beginning of the stance phase, are often seen during walking in patients with foot drop (Knutsson 1981; Dietz et al. 1994; Burridge et al. 2001). Thus, randomly activating the CP nerve-ankle extensor inhibitory pathways may not be necessarily useful in those patients with a weak soleus activation. However, regular, repeated stimulation of the CP nerve with functionally appropriate timings (i.e., functional electrical stimulation) might lead to improved locomotor control in chronic hemiplegic patients.

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8. LONG- AND SHORT-TERM EFFECTS OF FES IN CHRONIC CNS LESIONS

8.1 Introduction

Functional electrical stimulation (FES) of residual neural pathways below the lesion has been used as a functional aid for patients with central nervous system (CNS) disorders. Although the primary purpose is to restore lost function at the moment of use, there have been many physiological changes reported after long-term use of FES. For instance, regular application of FES to the lower limb has been shown to increase muscle strength, fatigue resistance, bone density, and muscle oxidative capacity (Stein et al. 1992; Greve et al. 1993; Rochester et al. 1995; Gerrits et al. 2000; Stein et al. 2002). Also, FES of the common peroneal (CP) nerve leads to improved walking speed with less physiological cost in hemiplegic patients (Stein et al. 1993; Taylor et al. 1999; Wieler et al. 1999; Barbeau et al. 2002). Recovery of locomotor function could partly be explained by these changes at the muscle level. However, the nervous system may also change at the spinal and/or supraspinal level over the period of FES-induced recovery (Crone et al. 1994; Barbeau et al. 2002; Miyai et al. 2002; Munchau et al. 2002; Frost et al. 2003).

In fact, there has been ample evidence indicating plasticity in the human adult CNS induced by stimuli to the periphery. A single session of repetitive peripheral nerve stimulation has been shown to induce the motor cortex reorganization and an increase of corticospinal excitability without increasing spinal reflex excitability (Hamdy et al. 1998; Ridding et al. 2000; Ridding et al. 2001; Kaelin-Lang et al. 2002; Khaslavskaia et al. 2002; Knash et al. 2003). Previously, we showed short-term effects of FES on corticospinal excitability in healthy subjects (Kido Thompson and Stein 2004). FES was

applied to the CP nerve during the swing phase of walking. After 30 min of walking with FES, corticospinal excitability for the TA, measured as the motor evoked potential (MEP) by transcranial magnetic stimulation (TMS), increased and this facilitatory effect lasted for at least 30 min. The increase of the TA MEP was quite different from the change in the silent period, which is known to, at least partly, reflect cortical inhibition (Fuhr et al. 1991; Chen et al. 1999; Werhahn et al. 1999), suggesting that FES did not simply increase general excitability of the cortex, but likely had specific effects on particular cortical neurons.

We have been investigating the effect of FES in hemiplegic patients in both short and long time frames to address the following questions: (1) Can a short-term application of FES increase corticospinal excitability in a patient with CNS lesion? (2) Does long-term use of FES induce cortical reorganization or alter the input-output curve? Here we report three different cases from an ongoing FES project to improve gait of persons with foot drop.

8.2 Materials and methods

8.2.1 Subjects

Participants of this study were adult chronic patients with CNS lesions who suffered from foot drop. All subjects gave informed consent for the purposes and procedures of the study, as approved by the Human Ethics Committee of the University of Alberta. Individuals with different pathologies were studied as long as all of the following criteria were satisfied: (1) medically stable for more than 6 months since the last episode of stroke or injury, (2) adequate ankle joint stability during standing, (3)

adequate cognitive and communication function to give informed consent; (4) able to ambulate with or without an assistive device, (5) inadequate dorsiflexion during the swing phase of gait, resulting in toe drag or inadequate limb clearance, (6) no lower motoneuronal damage at the lumbosacral level.

8.2.2 Functional electrical stimulation

WalkAide2 (Stein 1998) is a single channel FES device that has been developed to prevent foot drop by stimulating ankle flexors during the swing phase of walking for patients with various CNS disorders. WalkAide 2 is triggered by a tilt sensor (Stein 1998), and stimulates the CP nerve near the head of the fibula at 25 Hz. Stimulation begins when the leg is tilted backwards at the end of stance and ends when the leg is tilted forward at the end of swing. The participant could wear an FES device every day from the morning to evening, and each step by the affected leg was assisted by FES. WalkAide 2 also features an "exercise mode", by which the same stimulation is given for up to 30 min without any external trigger, and thus, can be used for therapeutic electrical stimulation. With "exercise mode", the CP nerve is stimulated at 25 Hz with 50% duty cycle (i.e., 2 s on and 2s off) regardless of the posture, so that, for example, a patient could use the device while sitting in a chair for training.

8.2.3 EMG recordings

The TA electromyogram (EMG) signals were collected using surface self-adhesive Ag-AgCl electrodes (Vermont Medical, Inc., Bellows Falls, VT). EMG recording electrodes were placed over the motor point for the TA with ~2 cm

interelectrode spacing. The signals were recorded with Axoscope (Axon Instrument, Union City, CA) at a sample rate of 5 kHz after amplification and band-pass filtering (10-1000 Hz). EMG signals were also rectified, low-pass filtered (RC filter, break point = 3 Hz), and displayed on an oscilloscope, so that the EMG activity level could be easily monitored by the subject during experiments.

8.2.4 Transcranial magnetic stimulation

MEPs were measured with the subject comfortably seated in a chair with the right or left foot fixed in a footrest. The hip, knee, and ankle joint angles were approximately 100°, 120°, and 110°, respectively. At the beginning of each experiment, MVC of the TA muscle was determined as the maximum rectified EMG level.

TMS, used to evaluate the corticospinal excitability, was delivered from an MES-10 Magneto-Electric Stimulator (Cadwell, Kennewick, WA) via custom-made, double-D coils with radii of 8 cm. The coil was held over the scalp such that the induced current flowed in the posterior-anterior direction in the brain. Under slight voluntary activation of the TA muscle (i.e., 15% MVC, see *EMG recording*), the MEP was elicited by TMS with an interval of 3-4 s between stimuli. First, the optimal TMS location, at which the lowest stimulus intensity was required to activate the ankle flexor, was explored by moving the coil over the scalp from 0 to 3 cm lateral to and –2 to 2 cm posterior to the vertex. Then, the input-output relation of the corticospinal pathway was obtained at the optimal location for the TA by increasing the TMS intensity from below the MEP threshold to the level at which the MEP reached its maximum amplitude. The TMS intensity, expressed as a percentage of the maximum current that can be discharged into the coil, was

increased in steps of 10%. Four MEPs were collected at each intensity. We analyzed both the peak-to-peak and mean rectified MEP (mrMEP) amplitudes, since in CNS disorder patients MEPs were generally more polyphasic and an input-output curve derived from rectified signals often seemed to give a better estimate of the input-output relation than that from peak-to-peak MEPs. Thus, the EMG signal was rectified, and after subtracting the mean background EMG level, mrMEP amplitude was calculated for a 15-30 ms period from the onset to the end of response. Averaged mr- and peak-to-peak MEPs were plotted against the stimulus intensity to estimate an input-output curve in real time by a custom written program in MATLAB (Mathworks Inc., Natick, MA). Subsequently, the motor output map was obtained using the TMS intensity of 10-20% above the threshold measured at the optimal location. A piece of thin flexible transparent plastic sheet with square grids was secured on the head, and the TMS coil was systematically moved over the scalp by 1 cm steps within -3 to +3 cm in anterior-posterior and -3 to +3 cm in medial-lateral direction in relation to the vertex. Although fitting square grids to the naturally curved surface of the scalp might introduce some errors (Thickbroom and Mastaglia 2002), considering the size of the TMS coil used and 1 cm of inter location spacing, we do not think that the use of square grids could change the obtained map shape dramatically. Typically, 0 to 3 cm contralateral to the target leg was examined, as our main interest was the reorganization in the hemisphere contralateral to the (more) affected leg. For better visualization of contour, the map was reconstructed after cubic interpolation using MATLAB. Following motor output mapping, the input-output relation was reexamined at the optimal location at which the average MEP amplitude was highest. All the MEP measurements were made during a constant

voluntary activation of the target muscle (i.e., 15% MVC of EMG activity level), so that the relative level of motoneuronal excitation remained the same between different days studied. When possible, the silent period (SP) was also measured as the period from the end of the MEP to the time, when background activity resumed in two of four responses (Knash et al. 2003; Kido Thompson and Stein 2004).

8.3 Results

8.3.1 Case 1: short-term effect of FES on corticospinal excitability

Subject R.T. was a 20-year-old hemiplegic male due to hemorrhagic stroke at the age of 7. He had regained locomotor function very well over the last 10 years, except for foot drop during the swing phase of walking. Prior to the application of FES, the optimal TMS location for the TA was determined, and the input-output curve was obtained during 15% MVC of the TA muscle contraction, as described in *Transcranial magnetic stimulation*. His MEP input-output curve at the optimal location (2 cm lateral and 0 cm posterior to the vertex) was similar to that of a healthy subject (Fig. 8-1A); the amplitude and threshold appeared to be fairly normal. He was young when he had a stroke and probably his CNS was still so plastic that the trauma to the cortex could be relatively well overcome. We tested the short-term effect of FES-assisted walking on corticospinal excitability in R.T. The basic protocol was the same as that used in the previous study (Kido Thompson and Stein 2004). R.T. was asked to walk on a treadmill for 3×10 min and the TA MEPs and SPs were measured before, between, and after periods of walking with FES. He could walk at 2.8 km/h for the whole 30 min.

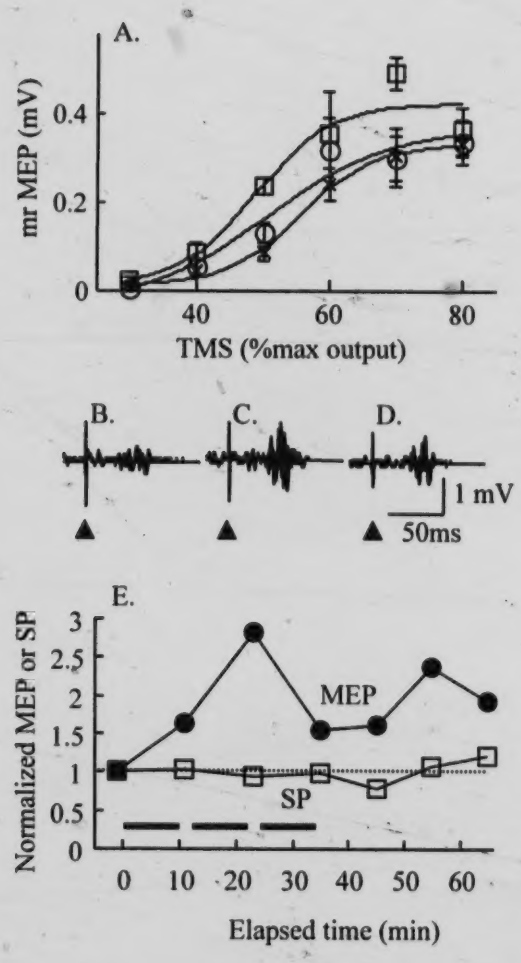

Fig. 8-1 The TA MEP evoked near the half maximum intensity increased after a single session of FES-aided walking in subject R.T. (young stroke patient). A: MEP input-output curves in the TA muscle are shown before (×), after 20 min (□), and after 30 min of walking (○) with FES (A). Each point shows the average mean rectified MEP (mean ± SE of 4 responses). The increase was most prominent in the middle of the curve (i.e., TMS intensities of 50% and 60%) after 20 min of FES. B, C, and D: MEPs elicited by TMS of 50% maximum output before (B), after 20 min (C), and 30 min (D) of walking with FES are shown. Four responses are superimposed for each measurement. E: Normalized mean rectified MEPs and SPs at TMS of 50% are shown along the experimental elapsed time scale. MEPs measured during 15% MVC of voluntary contraction were normalized by the initial values. Thick horizontal bars from 0 to 35 min show 3×10 min of walking with FES. Circles are for MEPs and squares for SP.

Fig. 8-1A shows the recruitment curves derived from mrMEPs before and after 20 and after 30 min of FES-aided walking. Similar to the finding in healthy subjects (Knash et al. 2003; Kido Thompson and Stein 2004), a prominent change was observed in the middle of the curve. In his case, the MEP amplitude increased dramatically after 20 min of walking, and remained moderately increased for the rest of experimental period. The TA MEPs at the middle intensity (i.e., 50%) before and after 20 and 30 min of FES are presented in Fig. 8-1B, C, and D, respectively. The mrMEPs and SPs at TMS of 50% were normalized by the initial values and shown in Fig. 8-1E. While the same TA background EMG was maintained throughout the experiment, the mrMEP amplitude increased by 183% after 20 min of FES-assisted walking (Fig. 8-1C and E), and this facilitation did not disappear for the rest of the experimental period (i.e., increased by ≅85% for up to 30 min after FES). As in the case of healthy subjects (Kido Thompson and Stein 2004), this increase of mrMEP at the middle intensity was not accompanied by the change in the SP (the mean ± SE of SPs over an experiment was 122.5±5.8 ms). In contrast, the MEP amplitude at the higher TMS intensity did not show consistent increase or decrease. The average change in mrMEP at TMS of 80% was 11±5% (mean ± SE) over the experimental period.

8.3.2 Case 2: effect of regularly applied repetitive CP nerve stimulation on corticospinal excitability

Subject J.T. was a 50-year-old male with incomplete paraplegia due to T12-L1 spinal cord injury resulting from a fall accident at a building construction site 11 years before the study. By the time of this study he had regained locomotor function well

enough, so that he could walk without any balancing aids, such as canes or crutches. However, disturbing foot drop during walking still remained, and he had worn an ankle foot orthosis (AFO) regularly. Also, because of his occupation, he needed to have ankle stability while standing during the day. Therefore, he chose to use an FES device for therapeutic stimulation, and kept wearing an AFO while he was at work. Basically, J.T. used WalkAide 2 "exercise mode" (see *Functional electrical stimulation*) on his more affected side (i.e., left leg) every evening at home for 1 hour at rest, and occasionally walked around the house with WalkAide on after the preset "exercise" stimulation was terminated. The motor output map for the left TA was obtained before and after one month of regular stimulation.

Fig. 8-2 shows his motor output maps before (A) and after (B) one month of stimulation. Both measurements were made using 60% of TMS maximum output during 15% MVC of TA activation, as described in *Transcranial magnetic stimulation*. Originally clear MEPs were mainly evoked anterior to the vertex, and only small responses were observed in the posterior part (Fig. 8-2A). This map shape was quite different from that of a healthy subject (see Fig. 8-2C); usually the peak of the map is at 1 cm lateral and 0-1 cm posterior to the vertex and MEPs in the anterior part appear to be smaller in healthy subjects (Knash et al. 2003; Kido Thompson and Stein 2004). The peak of the map in J.T. was at 2 cm lateral and 1 cm anterior to the vertex, likely around supplementary to pre- motor cortex (Rizzolatti et al. 2002). The uniqueness of his map shape was not because of failure in maintaining the same background level, since the TA background EMG changed only within ±7% (SE) from its mean level during mapping. Also, it was not due to mislocation of the vertex, as the map derived from stimulating the

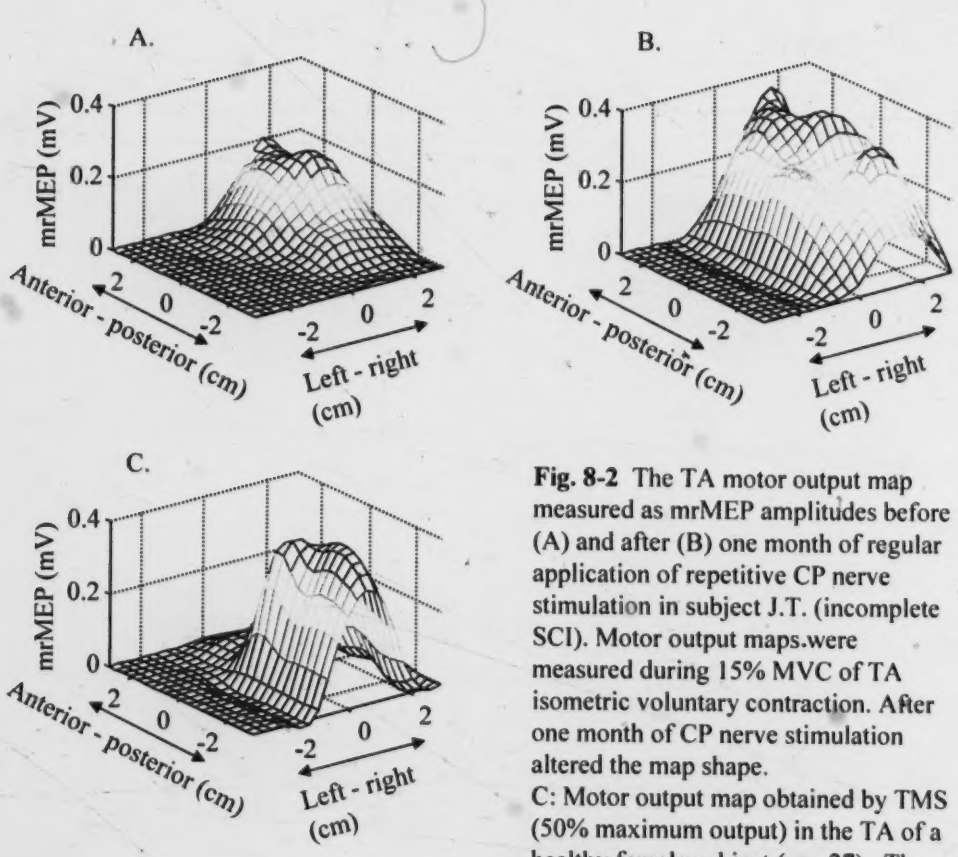

Fig. 8-2 The TA motor output map measured as mrMEP amplitudes before (A) and after (B) one month of regular application of repetitive CP nerve stimulation in subject J.T. (incomplete SCI). Motor output maps.were measured during 15% MVC of TA isometric voluntary contraction. After one month of CP nerve stimulation altered the map shape.
C: Motor output map obtained by TMS (50% maximum output) in the TA of a healthy female subject (age 27). The largest MEPs were evoked at 1 cm lateral and 0-2 cm posterior to the vertex.

other side of the scalp (i.e., map measured in the right TA) had a clear peak at 1 cm lateral and 1 cm posterior to the vertex. Thus, as has been suggested (Miyai et al. 2002; Munchau et al. 2002; Frost et al. 2003), it might be the case that corticospinal projection from the premotor (and supplementary motor) cortex had become more prominent to compensate for the weakened/damaged projection from the primary motor cortex over the period of spontaneous recovery.

One month of regular application of CP nerve stimulation altered the map shape (Fig. 8-2B). While the main peak of the map was the same as before, at 2 cm lateral and 1 cm anterior to the vertex, there were several locations posterior to the vertex where larger MEPs could be elicited. A new small peak could be found at 2 cm lateral and 1 cm posterior to the vertex, and another smaller peak near the vertex. Again, fluctuation of the background EMG during mapping was negligible, ±4% (SE) from its mean level. Together with the main peak, these new small peaks seem to surround a depression or valley centered at 1 cm lateral and 1 cm posterior, which is the usual peak location for the TA MEP in healthy subjects. It is also quite noticeable that the mrMEP amplitude is generally larger in Fig. 8-2B than in 2A, suggesting improved corticospinal excitability after one month. However, his MVC level of the absolute TA EMG amplitude changed dramatically between these two measurements (i.e., three times larger after one month). Thus, the change in map amplitude might be more or less due to the increased background EMG amplitude. Nonetheless, both maps were measured during the same relative facilitation level (i.e., 15% MVC), and the change in map shape would not attribute to the change in absolute EMG amplitude. New small peaks that started appearing in the posterior portion of the hemisphere after one month of stimulation likely

indicate that there are some new areas near or within the motor cortex that have become available for the TA activation.

8.3.3 Case 3: long-term effect of FES on corticospinal excitability

Subject M.L. was a 43-year-old male with incomplete quadriplegia as a result of head injury at the age of 24. According to his medical record, the original site of injury extended from the parietal-occipital lobe to the cerebellum, although detailed information about the trauma was not available. After the first 6 months of paralysis, he showed gradual motor functional recovery over a few years, and he had lived independently for the last few years. At the first assessment for the FES trial, he could walk with crutches but the very low speed (5.2m/min) prevented him from being a functional walker. Also, obvious left toe drag during the swing phase seemed to slow down his walking even more. As a result, for most daily activities he was using a wheelchair propelled by an arm and the less affected (i.e., right) leg. Thus, the setting of his FES unit was to activate his left ankle flexor muscles.

Over the 7 months of FES trial his walking speed was significantly improved. Initially, his walking speed was 5.6m/min with FES and 5.2m/min without FES. After 7 months the speed was 8.2m/min with FES and 7.7m/min without FES. Along with the improvement of locomotor function, the number of steps per day also increased from 470 steps/day to 1800 steps/day over the period of trial. Our physiotherapist noticed improved balance during walking near the end of the trial, while M.L. himself had became more confident with his locomotor ability and endurance. These functional improvements were also reflected in the increased TA MEP amplitude after long-term

use of FES (Fig. 8-3A and B). Effects of long-term FES application was investigated at 6.5 months point from the first day of FES use. Before and after the FES trial the motor output map was measured during the TA voluntary activation (i.e., 15% MVC level). M.L.'s MVC level of TA EMG amplitude did not change between the two measurements, so any changes in map amplitude or shape cannot be explained by the difference in absolute background EMG amplitude. Also, the background EMG during mapping was quite stable; SE was 4% of its mean level for the map before FES and 6% for that after FES. The intensity of 80% TMS maximum output was used for mapping both before and after FES trial. In M.L.'s case, peak-to-peak MEP amplitudes were shown in these maps, because MEPs were very small and maps constructed from mrMEPs basically showed similar results.

Before FES, his motor output map amplitude was generally small and peaks were hardly noticeable, if there were any (Fig. 8-3A). After long-term application of FES, the map amplitude increased; at the peak of the map the MEP amplitude was 2.5-3 times larger after FES (Fig. 8-3B). Moreover, there were 2 clear peaks, which could not be found before FES. One was centered at 2 cm lateral and 1 cm posterior to the vertex, and another at 1-2 cm lateral and 1 cm anterior to the vertex. These peaks emphasize the valley around 1 cm lateral and 0-1 cm posterior to the vertex, which is, as mentioned in the case 2, the usual peak location for the TA motor map in healthy subjects.

The TMS input-output curves before and after FES examined during 15% MVC of the TA activation are shown in Fig. 8-3D. TMS was applied at 3 cm lateral and 1 cm posterior to the vertex, at which the mean MEP amplitude was highest in the map obtained before FES (Fig. 8-3A). Prior to the FES trial MEP amplitudes were generally

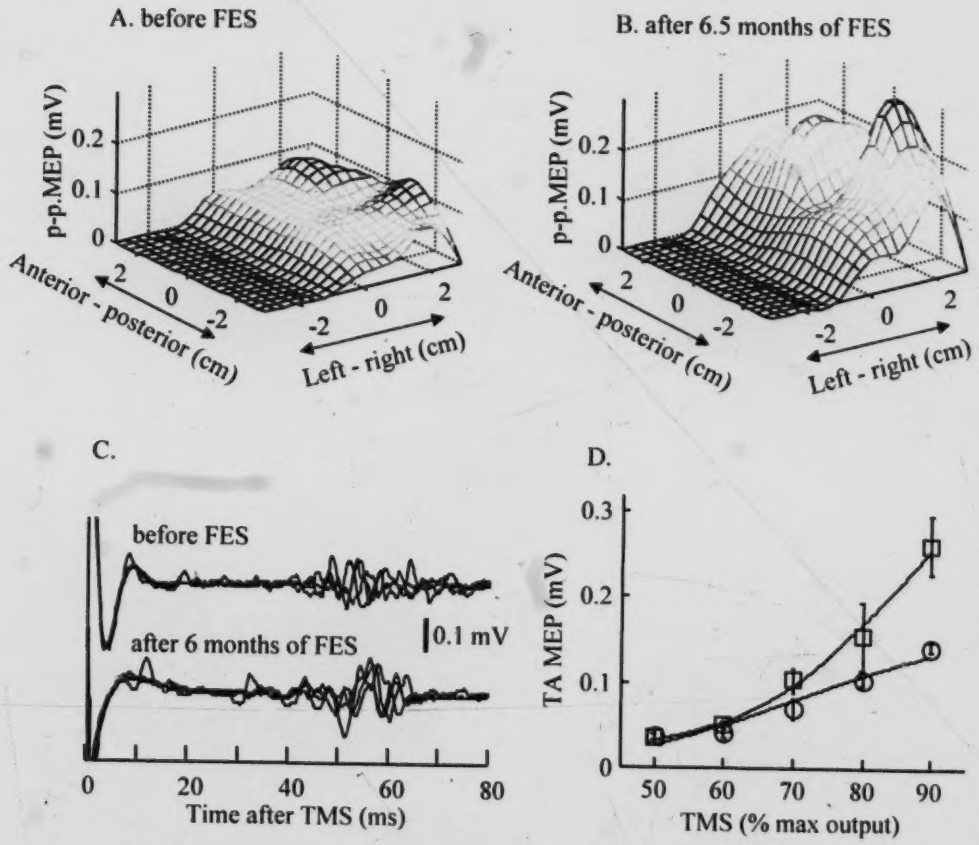

Fig. 8-3 The TA MEP amplitude increased after 6.5 months of FES use in subject M.L. (head injured patient). A and B: Motor output maps were measured during 15% MVC of TA isometric voluntary contraction before (A) and after (B) long-term FES. C: MEPs evoked at TMS of 90% output before and after long-term application of FES. Four responses are superimposed for each measurement. D: TA MEP input-output curves are shown before (○) and after 6 months of FES trial (□). Each point shows the average peak-to-peak MEP (mean ± SE of 4 responses). C and D were measured at the TMS location 3 cm lateral and 1 cm posterior to the vertex.

very low, compared to those elicited in healthy subjects using a similar protocol (Knash et al. 2003; Kido Thompson and Stein 2004). An increase of MEP amplitude in relation to an increase of TMS intensity was so little that we could not obtain the whole input-output "curve" or plateau part, as has been found in spinal cord injured patients (Davey et al. 1999). After 6.5 months of FES use, the slope of the input-output curve became steeper, although a plateau still could not be reached with a range of stimulus intensities we used. Fig. 8-3C shows MEPs elicited by 90% of TMS maximum output before and after FES. The increase in MEP amplitude after FES was clear. Note that the TMS location for the input-output curve (i.e., 3 cm lateral and 1 cm posterior) was no longer the optimal location after long-tem FES (see Fig. 8-3B); at the optimal site, the mean peak-to-peak MEP amplitude evoked by TMS of 80% was 0.31 mV.

8.4 Discussion

In the present report we showed three different cases to address the following issues: (1) whether a short-term application of FES increases corticospinal excitability in a patient with CNS lesion and (2) whether the long-term use of FES induces cortical reorganization or alteration of input-output curves. In the first case, we tested a short-term effect of FES in a young stroke patient and found increased corticospinal excitability after 30 min of FES-aided walking. In the second case, the effect of regularly applied CP nerve stimulation was investigated in a chronic spinal cord injured patient. Although the stimulation was not being used functionally during walking, the stimulus pattern was the same as that of FES. Nonfunctional, patterned repetitive CP nerve stimulation can increase corticospinal excitability for the TA in healthy subjects (Khaslavskaia et al.

2002; Knash et al. 2003), and recently we have shown that a similar increase of corticospinal excitability could be induced by a short-term application of FES (Kido Thompson and Stein 2004). Thus, as FES and repetitive CP nerve stimulation seemed to have similar effects in terms of increasing corticospinal excitability for the TA in healthy controls, investigating the long-term effect of repetitive CP nerve stimulation was thought to give us some ideas about the long-term effects of FES. After one month, in the motor output map there were a few new areas at which the TA of the stimulated leg could be activated. In the third case, the long-term effect of FES was studied in a long-standing head injured patient. Over the 6-7 months of FES trial, his walking was greatly improved and the TA MEP amplitude increased dramatically. The shape of motor output map was also changed; after FES we found a few new clear peaks in the map, presumably near the site for the primary motor area. All these observations are a part of ongoing FES project for improving hemiplegic gait, and more new cases will be reported in the future.

8.4.1 Short-term effect of FES in chronic hemiplegia

In the present study, corticospinal excitability for the TA increased after 30 min of FES-aided walking in a young chronic stroke patient (case 1). Similar to findings in healthy subjects (Knash et al. 2003; Kido Thompson and Stein 2004), a prominent increase of MEP was in the middle of the input-output curve but not at the intensity for the original maximum MEP. These suggest that a short-term application of FES likely facilitates preexisting corticospinal connections, but does not create new synaptic connections within the short time frame in a chronic hemiplegic patient. Also, the increase in MEP amplitude was not accompanied by the change in SP duration, indicating

that FES did not simply enhance both excitatory and inhibitory connections, but more specifically facilitated excitatory connections. The SP is thought to, at least partly, reflect cortical inhibition (Fuhr et al. 1991; Ziemann et al. 1993; Chen et al. 1999; Werhahn et al. 1999), and reduced intracortical inhibition has an important role in inducing plastic changes in the cortex (Jacobs and Donoghue 1991; Ziemann et al. 1998a; Ziemann et al. 1998b; Ridding and Rothwell 1999; Butefisch et al. 2000; Chen et al. 2002; Kaelin-Lang et al. 2002; Levy et al. 2002). Thus, changed MEP and unchanged SP after FES would suggest induction of corticospinal plasticity in a chronic hemiplegic individual. This finding is encouraging for other hemiplegic individuals using FES devices, since the short-term effect observed here might be accumulated when reintroduced in successive days (McKay et al. 2002).

8.4.2 Long-term effect of FES in chronic CNS lesions

In the present study long-term effects of regularly applied stimulation were reported in two different cases. In one of them, the effect of regularly applied CP nerve stimulation was investigated in a chronic spinal cord injured patient (case 2). As mentioned above, examining the effect of this FES-like stimulation may provide us with some clues for the long-term effects of FES (Khaslavskaia et al. 2002; Knash et al. 2003; Kido Thompson and Stein 2004). After one month of FES-like stimulation, the shape of the motor output map for the TA of the stimulated leg changed and new areas were available for the TA activation. The motor map shape was also changed in a chronic head injured patient after 6.5 months (case 3). Thus, both FES and FES-like stimulation induced a change in map shape, indicating cortical reorganization (Ridding and Rothwell

1997; Thickbroom and Mastaglia 2002). In both cases, MEP amplitudes of the map peaks also increased, suggesting increased corticospinal excitability. However, in the patient of case 2, we cannot deny the possibility that the increased MEP amplitude might be due to the increased absolute background EMG amplitude. On the other hand, in the subject of case 3, two different days of MEP measurements were made with the same background level (i.e. the same absolute background EMG amplitudes), and therefore, the increase of MEP amplitude at map peaks could not be explained by different background amplitudes. Moreover, input-output curves at the same stimulus location with the same background facilitation were different before and after FES. MEP amplitudes were larger at a given intensity after FES. Thus, in case 3, corticospinal excitability increased after long-term use of FES. Unfortunately, in this case, as the TMS output was not strong enough to elicit the plateau part of input-output curve, whether long-term FES led to creating new synaptic connections or preexisting connections become more accessible could not be investigated.

Does FES or FES-like stimulation lead to recruitment of particular cortical areas for the TA activation? In the subject of case 2, before regular application of FES like stimulation, most MEPs elicited were anterior and lateral to the vertex in the examined hemisphere (Fig. 8-2A), presumably over supplementary to pre- motor cortex. That is, these areas had already been recruited over years of spontaneous recovery. In case 3, after long-term use of FES a map peak was seen in the similar area, anterior and lateral to the vertex (Fig. 8-3B), again, probably resulting from activation of supplementary to pre-motor cortex. After FES or FES-like stimulation, in the motor output map we found a fairly large (case 2, Fig. 8-2B) or a larger (case 3, Fig. 8-3B) peak posterior and lateral to

the vertex, likely within the primary motor cortex near the usual TA hot spot. Thus, our present observations agree with previous studies (Miyai et al. 2002; Munchau et al. 2002; Frost et al. 2003) that functional recovery and cortical reorganization likely occur at multiple cortical locations including primary, supplementary, and pre- motor cortices. Furthermore, findings by Miyai et al. (2002) and Frost et al. (2003) and our cases might suggest that functional recovery involves strengthening by use of corticospinal connections from premotor areas, while reorganization within the motor cortex may be triggered by externally added stimulation, such as FES. This, however, needs to be further investigated.

Is there a relation between improvement in locomotor function and increased corticospinal excitability or cortical reorganization? We did not test the extent of functional improvement in subject J.T. (case 2). Subject M.L. (case 3) showed improvement in both locomotion and corticospinal descending connection. So far, from our current observations, a causal relation cannot be proven. Yet, since corticospinal pathways are recruited for the activation of the TA during walking (Schubert et al. 1997; Capaday et al. 1999; Schubert et al. 1999; Petersen et al. 2001), improving corticospinal connection (so that this pathway is more readily available) would be helpful for restoring/improving locomotor function. Currently, it is not clear whether the stimulation given with functionally appropriate timings (i.e., FES) is more effective than nonfunctional repetitive stimulation for improving locomotion or enhancing corticospinal connectivity is the key for locomotor improvement (i.e., stimulation does not have to be FES).

There is another issue to be addressed in the future. Spinal reflexes are often impaired in individuals with CNS lesions. An exaggerated stretch reflex, a common feature of spasticity (Delwaide 1987; Brown 1994; Hiersemenzel et al. 2000), and abnormal spinal reciprocal (Ashby and Wiens 1989; Crone et al. 1994; Boorman et al. 1996; Okuma et al. 2002; Crone et al. 2003) and presynaptic inhibition (Yang et al. 1991; Nielsen et al. 1995; Morita et al. 2001) are known to interfere with motor control in different ways. Besides, Levin and Hui-Chan (1992), Granat et al. (1993), and Solomonow et al. (1997) have shown that the long-term use FES can reduce spasticity, implying that some changes were induced at the spinal level. Thus, it seems necessary to study whether different spinal excitatory and inhibitory reflexes are altered after long-term use of FES, and if so, the extent of change in excitability of different spinal pathways.

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9. GENERAL DISCUSSION

Our somatosensory nervous system goes through continuous plastic changes in the short- to long-term with or without any specific external manipulations. This thesis studied modulation and modification of neural connectivity in the central nervous system (1) occurring through our daily activities and healthy aging processes, and (2) induced by damage to the CNS or external manipulation of afferent and efferent signals.

9.1 Modulation and modification of neural connectivity occurring through our daily activities, without any specific manipulations

9.1.1 Modulation of spinal reflexes

Different spinal reflexes are modulated by posture, task, and phase during locomotion in humans. For instance, task-dependence of the soleus H-reflex is well known. The reflex during walking is much smaller (i.e., often less than 1/3) than during standing (Capaday and Stein 1986) and the reflex gain further decreases from walking to running (Capaday and Stein 1987) even at the same speed (Edamura et al. 1991). During walking the H-reflex amplitude is clearly phase-dependent (Capaday and Stein 1986; Lavoie et al. 1997; Ethier et al. 2003), not merely due to the background motoneuronal excitability (Schneider et al. 2000) or activation of the antagonist muscle (Yang and Whelan 1993), but most likely due to presynaptic inhibition of the primary afferents. The task-dependency can be extended to the posture-dependency of the H-reflex amplitude; the amplitude generally decreases from lying prone to standing (Koceja et al. 1995; Angulo-Kinzler et al. 1998; Chalmers and Knutzen 2002). Passive changes in joint angle also alter the reflex amplitude (Brooke et al. 1995).

There are other spinal reflexes that are dramatically modulated from time to time, task to task. Over the step cycle of locomotion, the sign of cutaneous reflex is completely reversed in the TA; excitation during the swing phase to inhibition at the swing-stance transition (Yang and Stein 1990; Duysens et al. 1992; De Serres et al. 1995). The cutaneous response in the TA is predominantly suppressive during standing, but becomes facilitatory during running (Duysens et al. 1993). Autogenic Ib inhibition originating from Golgi tendon organs is also greatly modulated from inhibition during standing to excitation during walking. Pearson and his colleagues found that the inhibition, during standing, could be reversed to an excitation during some phases of walking in cats (Pearson and Collins 1993; Pearson 1995; Pearson et al. 1998).

In contrast, reciprocal inhibition is never reversed to facilitation across different tasks. Although there is some evidence for a task dependence of reciprocal inhibition, it appears to be weaker than for other spinal reflexes. As discussed in chapter 2, Ia, Ib, and cutaneous afferent signals actively contribute to generation and continuation of stable gait (e.g., for stretch and H-reflexes, Capaday and Stein 1986; Capaday and Stein 1987; Yang and Whelan 1993; Schneider et al. 2000; for cutaneous reflex, Yang and Stein 1990; Zehr et al. 1997; Zehr et al. 1998; Zehr and Stein 1999; for Ib afferent effects, Hiebert et al. 1995; Pearson 1995; Dietz and Duysens 2000; Pang and Yang 2000; see also Katz and Pierrot-Deseilligny 1999, for recurrent collateral, and Sinkjaer et al. 2000; Grey et al. 2001, for group II afferents), and thus, possess strong rationales for task- or phase-dependent modulation. A similar functional role for a task-dependence has not been found in spinal reciprocal inhibition. Nonetheless, in chapter 2 there was a consistent decrease of inhibition with speed (and thus, with task to a lesser extent). In fact, it seemed

reasonable to have less inhibition with faster speeds, since rapid shortening of the agonist would powerfully stretch the antagonist muscle spindles during faster locomotion, and reciprocal inhibition from the antagonist muscles would limit the activation of the agonist.

Overall, different excitatory and inhibitory spinal reflexes are task- (or speed-) dependently modulated to different degrees under supraspinal influence. The extent and direction (i.e., inhibitory or excitatory) of modulation might be related to the function that a given reflex pathway serves during different tasks.

9.1.2 Effects of healthy aging

Aging exerts significant influences over the neuromuscular system at multiple sites. Chapter 3 studied excitatory and inhibitory spinal reflexes in healthy human subjects having a wide range of ages. The results suggested a general decrease in excitability of spinal reflex pathways with age.

At the muscular level, a loss of muscle fibers (in both the number and size), especially a significant loss of fast twitch (type II) fibers is well known, while slow twitch (type I) fibers are relatively well preserved (Ishihara et al. 1987; Doherty et al. 1993; Luff 1998). Motor units are lost with age, preferentially from larger motoneurons with lower oxidative capacity (Campbell et al. 1973; Ishihara et al. 1987; Brown et al. 1988; Luff 1998). Thus, among surviving motor units, slow-twitch type units increase in proportion, due to partially compensating reinnervation occuring after denervation (Campbell et al. 1973; Doherty et al. 1993). Also in the intrafusal fibers, the dynamic spindle sensitivity decreases much more significantly with age than the static sensitivity

(Miwa et al. 1995). These explain a general reduction of muscle force, but cannot fully account for decreased reflex amplitudes, as studied in chapter 3.

Age-related changes in corticospinal excitability have been reported in several animal studies, such as decreased neuronal excitability in the motor cortex in aged rabbits (Mednikova Yu and Kopytova 1994) and reduced axonal conduction velocity of the aged cat pyramidal tract neurons (Xi et al. 1999). In humans corticospinal excitability and conduction may be slightly decreased with age (Rossini et al. 1992), but not to a comparable extent to spinal reflexes or muscle fibers. Terao et al. (1994) performed a quantitative analysis of myelinated fibers in the lateral corticospinal tract in 20 patients (aged 19-90) who died of non-neurological diseases. Examination of distribution of myelinated fibers showed that here was a significant age-related decrease in number of small-diameter fibers in lateral corticospinal tract at C6, T7, and L4 levels, while the number of large-diameter fibers was relatively well maintained at all ages. Recently, Pitcher et al. (2003) investigated the input-output characteristics on the motor cortex in young and old subjects using transcranial magnetic stimulation, and found no effect of age on the resting threshold, the maximum motor evoked potential, or the slope of input-output curve. Although the trial-to-trial variability of MEP amplitude was greater in older subjects at a lower stimulus intensity, overall results indicated well preserved corticospinal excitability in aging.

Aging gradually alters multiple sites in the neuromuscular motor system from the cortex to muscle. However, the extent of change does not seem to be the same across different levels, or even within the same level (e.g., differences between the type I and II muscle fibers, and between the large and small diameter corticospinal tract neurons). Of

special interest the corticospinal tract remains well accessible for motor control in old individuals. While reflexive control of muscle activation generally becomes weaker with age, voluntary (i.e., corticospinal) control of activation of spinal motoneurons does not. This supports the presence of plasticity in the human adult nervous system, since the corticospinal tract is the key for learning of a simple behavior at the spinal level (Wolpaw and Tennissen 2001; Chen et al. 2002; Chen and Wolpaw 2002) and corticospinal neurons are themselves highly plastic as shown in chapters 4, 5, and 8.

9.2 Modulation and modification of neural connections induced by external manipulations

9.2.1 Damage to the central nervous system

The most powerful and irreversible manipulation to the nervous system is a lesion, such as spinal cord injury and stroke. Lesions in the CNS strongly modify spinal reflexes and corticospinal descending inputs to the spinal cord.

As mentioned in chapter 7, different spinal reflexes are differently impaired after CNS lesions (presynaptic inhibition, Yang et al. 1991; Stein et al. 1993b; Faist et al. 1994; Nielsen et al. 1995; Katz 1999; Aymard et al. 2000; Morita et al. 2001; stretch or H-reflex, Taylor et al. 1984; Delwaide 1987; Thilmann et al. 1991; Brown 1994; Ib inhibition, Delwaide and Oliver 1988; however, see also Downes et al. 1995; recurrent inhibition, Katz and Pierrot-Deseilligny 1982; Shefner et al. 1992; Katz and Pierrot-Deseilligny 1999; reciprocal inhibition, Ashby and Wiens 1989; Boorman et al. 1991; Crone and Nielsen 1994; Boorman et al. 1996; Okuma and Lee 1996; Okuma et al. 2002; Crone et al. 2003). In most studies, the observations have been made within the first one

year or two after CNS lesions. However, some patients experience slow, gradual functional recovery over a few years, and there is no reason to believe that altered spinal reflexes due to damage to the CNS cannot be modified during the functional recovery over several years. Thus, chapter 7 studied spinal reciprocal inhibition of ankle extensor or flexor muscles in long-standing ambulatory subjects with foot drop. Some had regained locomotor ability within a year from accidents, whereas some others over a few years. Clearly existing foot drop during walking in all of these subjects led to hypothesizing decreased reciprocal inhibition of the soleus and increased inhibition of the tibialis anterior (TA) muscle. To our surprise, inhibition of the soleus was relatively small in subjects ≤1 year after the onset of CNS damage, but strong at later times, while inhibition of the TA was present less than 1.5 year after onset of CNS lesions, but progressively decreased and was eventually replaced by excitation.

Lesions to the CNS alter corticospinal connectivity. Motor evoked potentials (MEPs) decrease in response to transcranial magnetic stimulation (TMS) (Davey et al. 1999, also Stein and Thompson, unpublished observations). Also, abnormal cortical inhibition, which is reflected in the cortical silent period (SP) after an MEP, has been found in patients with CNS lesions. Classen et al. (1997) performed a detailed investigation on a prolongation of the SP in acute stroke patients with clearly prolonged SP durations with normal size of MEPs in the first dorsal interosseus muscle. TMS investigations continued for up to 2 years post-stroke showed that the SP duration decreased in parallel with clinical improvement in all patients. Thus, the relation between motor dysfunction and hyperactivity of cortical inhibitory interneurons was suggested in stroke patients. In acute stroke patients, Liepert et al. (2000b) also found prolonged SP

duration with reduced intracortical inhibition, indicating disinhibition of the motor cortex. In contrast, loss of the SP was found in patients with spinal cord lesions (Shimizu et al. 2000). Can the intracortical or corticospinal connectivity be modified along the functional recovery? Cortical and corticospinal plasticity in CNS lesions was dealt with in chapter 8.

9.2.2 Manipulation of the peripheral nerve activity

Alteration in peripheral afferent (and efferent) activity by means of peripheral nerve stimulation (Hamdy et al. 1998; Ridding et al. 2000; Ridding et al. 2001; Kaelin-Lang et al. 2002), transient deafferentation (Brasil-Neto et al. 1992; Brasil-Neto et al. 1993; Ziemann et al. 1998a; Ziemann et al. 1998b; Werhahn et al. 2002), or intensive use (i.e., use-dependent plasticity, (Butefisch et al. 2000; Liepert et al. 2000a; Ziemann et al. 2001) modify corticospinal connectivity.

In chapter 4, we also showed that only 30 min of repetitive stimulation of the common peroneal (CP) nerve increased corticospinal excitability for the TA. The maximum MEP amplitude, however, did not change, indicating the total connectivity was not changed with a short-term application of the peripheral stimulation.

9.2.3 Functional electrical stimulation

Functional electrical stimulation (FES), in which increased use of particular muscle groups and peripheral nerve stimulation are combined together, induces long-term effects at the muscular level (Stein et al. 1992; Greve et al. 1993; Rochester et al. 1995; Gerrits et al. 2000; Stein et al. 2002) and functional recovery (Stein et al. 1993a; Burridge

et al. 1997; Barbeau et al. 1998; Taylor et al. 1999; Wieler et al. 1999; Ladouceur and Barbeau 2000; Barbeau et al. 2002). However, effects of FES on the nervous system have not been systematically studied. Thus, the short- and long-term effects of FES on the CP nerve were investigated in this thesis.

In chapter 5, short-term effects of FES of the CP nerve during walking on corticospinal excitability were studied in healthy subjects. This study was a prelude to examining the long-term effects of FES in patient populations, since individuals suffering from foot drop due to CNS lesions have been treated in our laboratory (Wieler et al. 1999). FES was applied to the CP nerve during the swing phase of the step cycle when the ankle flexors are active, and MEPs by TMS were measured in the TA. Thirty minutes of walking with FES increased the MEP in the TA, similar to the finding in chapter 4, whereas without FES (i.e., walking itself) did not. FES-induced facilitation of the corticospinal response was not accompanied by an increase of reflex excitability of the TA at the spinal level (chapter 6), indicating enhancement of cortical (rather than spinal) excitability by FES. On the other hand, walking with FES of the CP nerve slightly decreased the soleus H-reflex, suggesting that a short-term application of FES may reduce unwanted exaggerated stretch reflex in a spastic patient population.

Finally, three different cases of short- or long-term application of FES or FES-like stimulation were shown in chapter 8. These are a part of ongoing FES project for improving hemiplegic gait, and more new cases will be reported in the future. All three patients participated in our study had foot drop and that was treated by FES of the CP nerve. Present observations suggest that FES or FES-like stimulation increases corticospinal excitability and/or induces cortical reorganization.

To summarize, this thesis studied modulation and modification of spinal reflexes and corticospinal connections in human adults. Without any external manipulation, different spinal reflexes are differently modulated from moment to moment, task to task, and also modified over a much longer time frame (i.e., the aging process). Damage to the nervous system, which powerfully alters the nervous system activity and connection, changes spinal reflexes, although a changed reflex response is also subject to continuous modification of neural connections. Corticospinal connectivity is also highly modifiable. Only a single session of short-term peripheral nerve stimulation or FES can change corticospinal excitability. Preliminary observations in the long-term FES project in patients with foot drop suggested that the long-term regular application of FES may improve corticospinal connections and induce cortical reorganization.

9.3 Future directions

The human adult nervous system is highly plastic, and the plasticity of our nervous system has been considered to be fundamental for improving or maintaining motor control in short to long time frames. However, most of the human studies have dealt with either a short-term plasticity or changes before and after a long-term manipulation (e.g., regularly applied training or stimulation), and relations between short- and long-term changes have not been clarified. In animal models, neural plasticity may involve alteration of efficacy of existing synapses, such as LTP (Kandel 2000; Rioult-Pedotti et al. 2000) and/or structural change of synapses (i.e., addition or subtraction of synapses) (Atwood and Wojtowicz 1999; Chklovskii et al. 2004). While understanding of plasticity at the neuronal level (i.e., connection between two neurons) has deepened,

plasticity of the system (i.e., several different pathways interacting with one another) has not been nearly as well understood as plasticity at synapses. It seems necessary to understand how long-term changes in the system are induced from short-term changes and how different elements of the nervous system are modulated/modified while plastic changes are occurring in the adult nervous system. The knowledge of how and where the neural plasticity occurs may facilitate development of therapeutic treatments for individuals with motor disabilities.

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